VOLUME 82 *September 1960* NUMBER 3

THE AMERICAN REVIEW OF RESPIRATORY DISEASES

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

OFFICIAL JOURNAL OF AMERICAN THORACIC SOCIETY
MEDICAL SECTION OF THE NATIONAL TUBERCULOSIS ASSOCIATION

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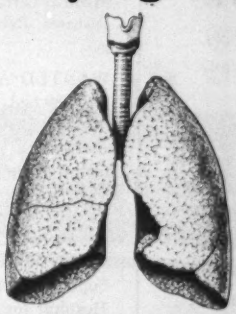

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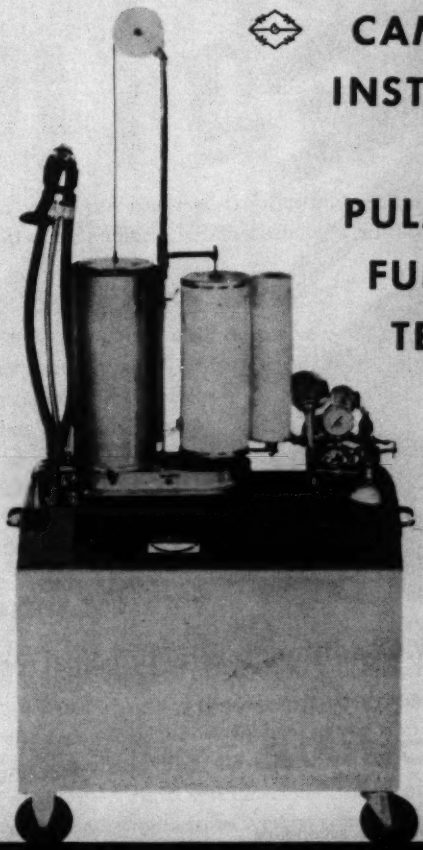

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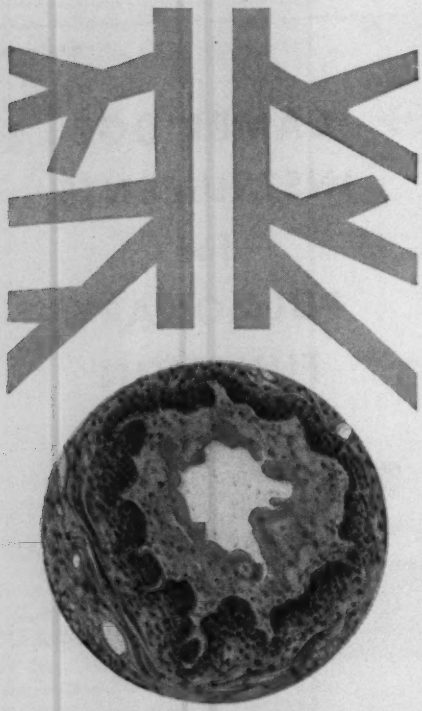

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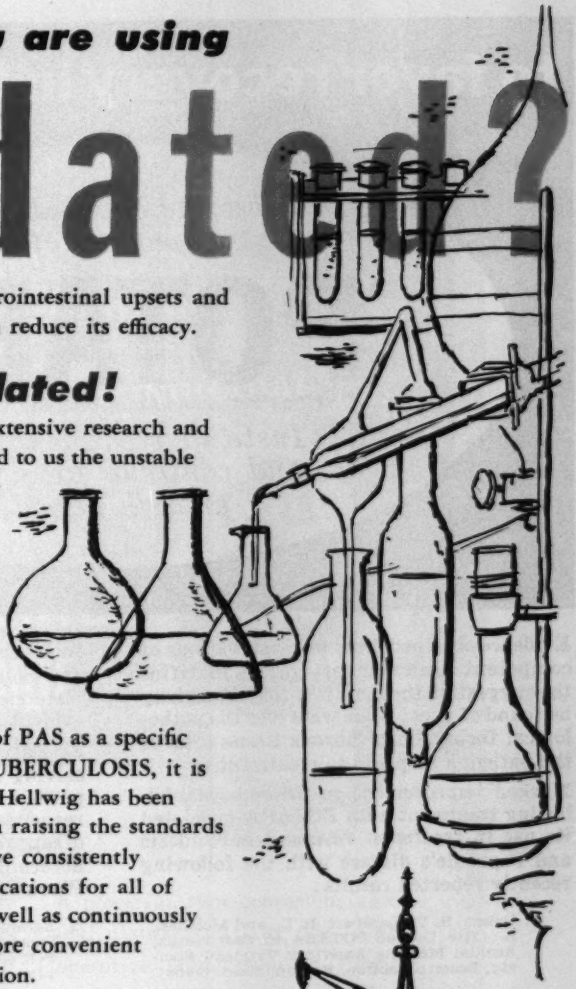

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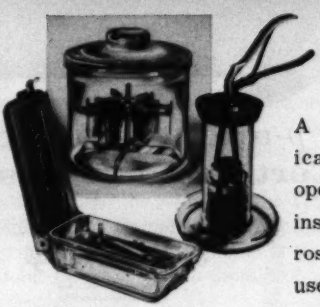

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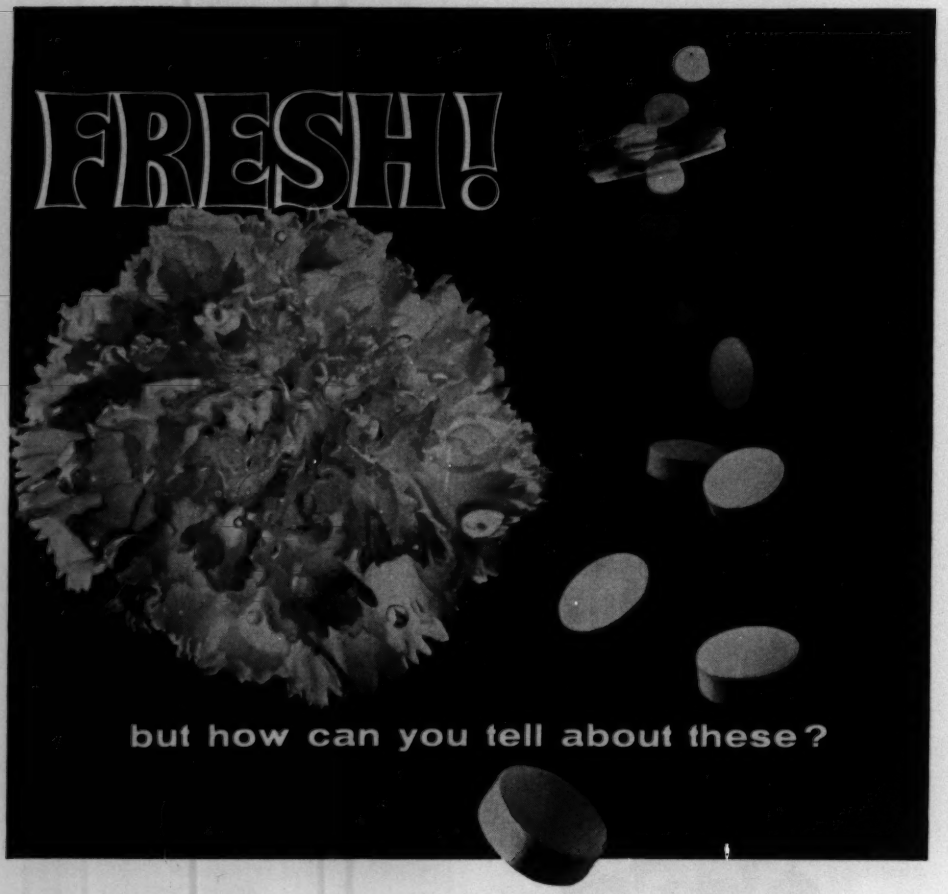

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VOLUME 82 *September 1960* NUMBER 3

THE CONQUEST OF TUBERCULOSIS

Keynote address of the Honorable Lister Hill, United States Senator from Alabama, at the opening of the 1960 Annual Meeting of the National Tuberculosis Association, Los Angeles, California, May 16, 1960

LISTER HILL

(Received for publication May 16, 1960)

How truly revolutionary is the concept of the conquest of tuberculosis can be appreciated only by those familiar with the history of the long and arduous medical effort to bring it under control.

Tuberculosis is an ancient enemy of mankind, as old as the history of civilization. We find it recorded on the tablets of the Babylonians.

Hippocrates, the father of medicine, wrote about "consumption." Ancient Greek medical literature abounded with speculations as to the cause of tuberculosis and Galen, the Greek physician who lived in the second century, mentioned the possibility of transmission of the disease from one person to another.

Throughout the Roman era and the Middle Ages, tuberculosis continued to spread death and heartbreak in its path.

It is hard for us today to realize the terror which for so many centuries this disease struck in the hearts of men. In the middle of the 17th century, when tuberculosis caused at least one out of every five deaths in England, it was branded by John Bunyan as "the captain of all these men of death."

Consumptives everywhere were pilloried and shunned. In Italy and Spain, edicts required that the disease be reported immediately and the belongings of its victims be destroyed. As late as the middle of the 19th century the immortal musician, Frederic Chopin, was driven to the docks in a wheelbarrow by his indignant neighbors when they discovered he had tuberculosis.

Amid the superstition, terror, and fear surrounding this age-old scourge of mankind, there were occasional shafts of light from the infant science of medicine. In 1722, Dr. Benjamin Marten declared that consumption might be caused by "minute living creatures," and he speculated that one day drugs might be found to kill these animalculae, as he called them. The physicians of his day pretty much ignored Marten's thesis as too revolutionary.

The modern scientific attack upon tuberculosis really had to wait upon the discovery in 1882 by the German physician, Robert Koch, of the incriminating agent—the tubercle bacillus.

While the discovery of the cause of tuberculosis was a tremendous scientific landmark, because of the lack of effective treatment the disease continued its ravages. Here in America at the turn of the century, tuberculosis outranked even influenza and pneumonia as the leading killer. In fact, as America became more urbanized and its city dwellers lived in overcrowded housing and were denied the benefits of nutritious diets and fresh air, tuberculosis increased alarmingly.

The physicians of the era felt a deep sense of helplessness. In the words of the medical historian, Richard Harrison Shryock: "The majority of regular physicians viewed the disease as hopeless as late as the end of the 1800's. It called only for the usual prescription of olive oil and a trip to Florida."

The modern public health movement against tuberculosis was inspired by the work of Dr. Edward Livingston Trudeau. Having contracted tuberculosis when nursing his brother, Trudeau journeyed to his beloved Adirondack Mountains in northern New York State to spend what his

physician led him to believe were the last few months of his life. However, the bed rest and fresh air restored Trudeau to health, and in 1885 he opened his small but soon to become famous tuberculosis sanatorium at Saranac Lake. Twenty-five years later, throughout the nation, there were approximately 400 tuberculosis sanatoriums.

Because of the extremely contagious nature of the disease, there was a desperate need at the time for an educational movement to persuade the American people to act to prevent the spread of the disease.

In 1892, Dr. Lawrence Flick, a consumptive himself, founded in Pennsylvania the first Society for the Prevention of Tuberculosis.

By 1904, there were 38 voluntary tuberculosis societies in the country and it was increasingly apparent that the movement had to be welded together nationally. Dr. William Osler of Johns Hopkins Medical School, and one of America's greatest physicians, chaired the first planning committee of fifteen physicians which included his equally eminent colleagues, Dr. William H. Welch and Dr. Trudeau. On March 28, 1904, at the historic College of Physicians building in Philadelphia the National Tuberculosis Association came into being. Dr. Trudeau was chosen as your first president.

The early years were trying ones. Not only was it hard to raise money for the educational campaign, but the basic job of overcoming public prejudice against the disease was an enormous one. In this most difficult of tasks, the early workers of the national tuberculosis movement set a magnificent pattern of public education which was to be emulated in later years by voluntary organizations in the field of poliomyelitis, cancer, heart disease, mental illness, and the many neurological diseases.

In spite of a public paralyzed by unreasoning fear, suspicion, and prejudice, the fight went on. In Chicago, Miss Kate F. O'Connor, known to all in the Windy City as "fresh-air Kate," began her campaign to air the streetcars of Chicago. Her chief enemies were irate streetcar conductors. In 1909, in Georgia, an anti-spitting bill was defeated by only five votes.

The use of the Christmas seal, started by a Danish postmaster and publicized in this country by the great reformer Jacob Riis, who had lost six brothers to tuberculosis, solved some of the financial problems of the new Association. In the decades that followed, the Association's effectiveness grew as it fought for more sanatoriums, enlarged public health campaigns, mass detection through chest X-rays, and other preventive measures.

With the isolation of streptomycin, which was first announced in January, 1944, by Dr. Selman Waksman, the drug attack against tuberculosis was launched. In succeeding years, the drugs PAS and isoniazid were added to the chemotherapeutic offensive.

The results were almost miraculous. In New York City, for example, one out of every four tuberculosis beds was eliminated and $36 million in planned construction of tuberculosis beds was called off.

In 1946, the Veterans Administration initiated its now world-famous evaluation of the effectiveness of the drugs against tuberculosis. As a result of intensive application of these new medications, the V. A. in the period from 1954 to 1959 cut the death rate among its tuberculous veterans by more than 50 per cent and eliminated the use of 6,000 beds for tuberculosis. Testifying before Congressional committees, V. A. officials estimated that this mass drug evaluation and treatment program had saved American taxpayers a minimum of $100 million.

As the over-all figures on the conquest of tuberculosis in America began to be pooled, those who knew of the centuries-long battle against the disease were filled with wonder.

Between 1948 and 1958, new cases of tuberculosis dropped 50 per cent. In 1957, only 12,000 people died from tuberculosis as compared with 50,000 who had died in 1945. When related to the fact that in the first half of the 20th century tuberculosis had claimed five million American lives, the low death rate in 1957 is seen in true perspective as a magnificent triumph of the National Tuberculosis Association and modern medical research.

Although tuberculosis has been dropped from the list of the leading killers of our time, it is still with us. Today, 250,000 Americans have active tuberculosis, and 100,000 of these are not known to health departments. More than half a million Americans have inactive tuberculosis and we know that half the newly reported active cases of tuberculosis are among people under 45. Furthermore, about 2,000,000 persons under 25

years carry tubercle bacilli in their bodies and are therefore a potential source of infection for many years to come.

Aware of these facts, in December, 1959, the National Tuberculosis Association and the United States Public Health Service joined forces at Arden House, New York, to plan a grand strategy dedicated to wiping out the remaining strongholds of tuberculosis in the United States. This is a splendid example of united effort by a voluntary health organization and by federal, state, and local health departments to bring the fruits of medical research to bear in an all-out attack upon a single disease.

Just ten days ago, in testifying before our Senate Health Appropriations Committee, Dr. Robert L. Yeager of your Board of Directors strongly endorsed the objectives of the Arden House Conference. And he threw out to all of us this challenge: "We, ourselves, must take the blame if the eradication of tuberculosis is not achieved within the foreseeable future in the United States."

In attempting to break the "chain of infection" of tuberculosis, you today have a set of medical weapons unheard of just two decades ago. In addition to the new drugs, you have a tuberculosis skin test for detection; you have the vaccine BCG which had proved so effective in many parts of the world.

You are supporting on-going medical research which should provide even more effective tools. In a most exciting development, you have joined with the Public Health Service in support of several projects designed to test the effectiveness of isoniazid as a prophylactic agent against tuberculosis.

The tests already have demonstrated that among pre-school children exposed to tuberculosis isoniazid can prevent up to 80 per cent of the serious complications of the disease.

Dr. James Shaw, Assistant Surgeon General of the Public Health Service, recently told our Senate Committee of the "rapid and favorable results" of experiments with isoniazid as a preventive among the Alaskan natives and among the Pueblo and Navajo Indians of the southwestern United States.

I was gratified and challenged to learn of the recent action by your Board of Directors through which the sphere of interest of your powerful volunteer Health Association will go beyond tuberculosis to include the baffling diseases of the respiratory tract. Upper respiratory diseases account for two out of every three cases of acute illness in our country, and lung cancer today causes more deaths in the United States than does tuberculosis. They run up an annual medical bill of some $3 billion. In the Asian flu epidemic of 1957–1958 alone, 75,000 American deaths were charged to influenza. Think of it! More Americans died in this one epidemic than were killed in all the battles of World War I. Surely, the energies and the resources of your Association are essential not only to reduce this fearful death toll but to alleviate this greatest cause of lost productivity to our nation.

Now let us look for a moment beyond the immediate frontiers of our nation. Although the last few years have witnessed the most rapid decline in tuberculosis mortality the world has seen, this ancient enemy is still prevalent in most of the underdeveloped countries. This was dramatically proved in the tuberculin testing done on 38 million children and young adults in the International Tuberculosis Campaign during the early 1950's. For example, that testing program showed that in Korea, India, Ceylon, and Formosa, from 65 to 80 per cent of the children aged fifteen were infected to some degree with tuberculosis.

We must be alert to the fact that an underprivileged two-thirds of the human race are constantly afflicted by the ravages of disease. In the underdeveloped areas millions of these people rarely, if ever, have a physician to treat their ills.

We must recognize that medicine is the universal language. It speaks to all peoples of all lands. We must make our medical voice heard by those who without us have no hope. We must say to them that we are concerned, that we do care for their lives and for their happiness.

I am convinced that medicine, with its power to cross all political frontiers and its ability to be understood by all men, can do more for world understanding and for permanent peace than can all the billions of dollars now being poured into the armaments race.

I am sure you shared my gratification when President Charles de Gaulle of France on his recent visit to Washington emphasized the pressing need to immediately extend the boon of better health and longer life to our less fortunate neighbors. It is most interesting that President

de Gaulle should have singled out as a possible starting point for such a program a campaign against tuberculosis in Asia.

Your own able and dedicated director, Dr. James E. Perkins, who is a leader in the International Union Against Tuberculosis, was among those instrumental in placing before the Executive Board of the World Health Organization a resolution urging the Organization to accept "as a top priority target" the elimination of tuberculosis throughout the world. This resolution is before the World Health Assembly now meeting in Geneva.

In 1958, in the interests of a world-wide attack against disease, I introduced legislation to provide the mechanism through which this country would join with all the countries of the world in a united medical research offensive against the major killers and cripplers of mankind. The bill provides for the establishment of a National Institute of International Health and Medical Research, the main purpose of which would be the support of medical research projects submitted by competent investigators from any part of the world.

In May of 1959, the Health for Peace Bill passed the Senate by an overwhelming majority. The House Subcommittee on Health has unanimously endorsed a similar bill. We must make sure that this legislation is enacted before the close of the present session of Congress.

There are those who would contend that, since so much of the research funds and facilities now at work in medicine are available only in our own country, international medical research would be for the United States another case of all going out and nothing coming in. This is not true. We have here no corner on medical knowledge. As Dr. Howard Rusk, director of rehabilitation at the New York University's Bellevue Medical Center and Medical Editor for the *New York Times*, has so ably stated the case: "Medicine . . . is the most beautiful evidence of internationalism that exists in the world." And he cited these examples:

"Microbiology came from Holland. Immunology with vaccination came from Great Britain. Antisepsis and asepsis came from Joseph Lister in Great Britain. Bacteriology came from Pasteur in France. Sulfonamides came from Germany—but they waited sixteen years because an assistant missed one compound, and it was found sixteen years later in a laboratory in another country. Penicillin came from Great Britain. Insulin came from Canada. Cortisone came from the United States. Anesthesia came from the United States. Rauwolfia came from India."

Dr. Rusk reminds us that, although we in America have much to give to the health and well-being of other peoples, we have much to gain for ourselves.

I do not have to tell you, the pioneers in the field of tuberculosis, of the magnificent vistas which can be opened up by a world-wide attack on tuberculosis. You who know so well the incomparable contributions of a Pasteur, a Virchow, a Jenner, a Roentgen, a Robert Koch, and a Joseph Lister to the modern attack upon tuberculosis need little persuasion as to the importance of an accelerated international effort drawing upon the best medical brains in all parts of the globe.

In his excellent history of the first fifty years of your organization, Shryock paid tribute to your enormous and continuing impact upon the public health of our people in these words:

"Initiator and outstanding example of the voluntary health movement in the Unites States, the National Tuberculosis Association has already written an important chapter in the social and medical history of the American people. Yet, this is only prelude; what has gone before will give form and impetus to the future."

My friends of the National Tuberculosis Association, I say to you who have blazed a trail and who have performed mighty works, this is the hour for men of medicine, men of science, men dedicated to the cause of relieving human suffering and saving human lives, to join in forging and in using the tools of health that they may be the tools of peace.

Let us accept the faith of the immortal Louis Pasteur, when he declared: "I hold the unconquerable belief that science and peace will triumph over ignorance and war . . . that nations will come together—not to destroy but to construct . . . and that the future belongs to those who accomplish most for humanity."

STUDIES OF BACILLARY POPULATIONS IN EXPERIMENTAL TUBERCULOSIS OF MICE TREATED BY ISONIAZID[1]

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INTRODUCTION

When mice are infected with a large dose of virulent tubercle bacilli and treated with isoniazid alone after an interval of fifteen days, resistant populations emerge very frequently. This fact, noted by Grumbach, is not confined to isoniazid, but is produced with many other antibacillary agents (1–3). Treatment of established murine tuberculosis under certain conditions thus furnishes a useful experimental model for the quantitative study of resistant populations appearing *in vivo*. Similar populations have been observed *in vivo* in other experimental circumstances (4–8); but up to now precise quantitative studies on their conditions of emergence and their composition are lacking. Even in man such studies are rare (9, 10). The fact that it is necessary to administer a single antituberculous agent to obtain experimentally resistant populations, when the treatment of human tuberculosis is usually with multiple-drug regimens, does not detract from the utility of such studies, as resistant populations appearing in these two circumstances only differ in one single character, their frequency.

The present work is a quantitative and qualitative study of populations of tubercle bacilli found in the lungs of mice during delayed treatment with isoniazid. Two different doses have been used, one large, the other small, and the evolution of bacillary populations has been studied in relation to the active isoniazid concentration found in blood serum with these two doses of isoniazid. The administration of large doses of isoniazid is of topical interest. The study of the concentration of active isoniazid in blood serum has given rise to much work, and will give rise to more. Although the principal aim of the present work is to draw attention to certain fundamental obscurities which persist in our knowledge of the mechanisms of appearance of great resistant populations, the practical implications will also be considered.

MATERIAL AND METHODS

Mice: All of the mice used in the present study came from the strain M. L., in current use at the Pasteur Institute, by which it was selected for its susceptibility to tuberculosis. The weight of the animals when inoculated was between 15 and 18 gm.

Strains of bacilli and mode of infection: Two strains of tubercle bacilli were employed for the infection, strains *S.13* and *S.17*, both recently isolated from patients with tuberculosis as yet untreated by drugs. These two strains were normally susceptible to isoniazid. Their virulence in mice was the same as had been found with strains not previously adapted to mice. With strain *S.13*, the T.50[2] for an intravenous inoculation of 0.5 mg. was twenty-six days; with strain *S.17*, twenty-two days.

One half of the animals were inoculated with strain *S.13*; the other half, with strain *S.17*. The distribution of the animals infected with the two strains was the same in the two treatment groups. The inoculation was made by the intravenous route from 8-day-old cultures in Dubos liquid medium. The inoculum was 0.25 mg. It contained 1.1×10^7 viable bacilli for the strain *S.13*, and 1.5×10^7 for strain *S.*17. Two strains were used because the initial objective of the experiment was partly to see if the populations obtained varied with the strain used. The two strains chosen for this purpose differed a little in the numbers of resistant mutants initially present. In addition, in these particular strains, the number of mutants was a little higher than in the strain H37 (table 1). In fact, no difference in the final populations was noted, and the results observed will be presented together.

Treatment: The inoculated animals were left fourteen days without treatment. From the fifteenth day, half of the animals were treated with a daily dose of 0.1 mg. of isoniazid; the other half, with 1 mg. At the beginning of the experiment, the regimens represented approximately 5 mg. per kg. and 50 mg. per kg. of isoniazid per day; at the end, with the increased weight of the animals, the dosage represented approximately 3 mg. per kg. and 30 mg. per kg., respectively. The drug was given in one dose, orally, by esophageal tube on six days a week. Treatment was continued for one hundred and forty days (twenty weeks).

Study of bacillary populations: An equal number

[1] From the Tuberculosis Research Laboratories, Pasteur Institute, Paris, France.

[2] T.50: the point in time at which one half of the animals had died.

TABLE 1

NUMBER OF COLONIES ON LÖWENSTEIN-JENSEN MEDIUM CONTAINING DIFFERENT CONCENTRATIONS OF ISONIAZID YIELDED BY AN INOCULUM OF 10^6 BACILLI OF THE WILD STRAINS S. 13, S. 17, AND H37Rv*

Strain	Concentration of Isoniazid (γ per ml.)							
	0.05	0.1	0.2	0.5	1	5	25	100
S. 13	10^4	300	23	8	10	7	2	2
S. 17	10^4	240	120	60	44	24	12	0
H37Rv	†	11	7	4	2	0.8	0.5	0

* For technical details see text.
† Not inoculated.

of animals from each treatment group—in general, 6, half of which were infected by the strain *S.13*, and half by *S.17*—was sacrificed on day 0, the fourteenth, thirty-fifth, seventieth, one hundred and fifth, and one hundred and fortieth days of treatment. The spleen and lungs were sampled. Each organ was homogenized and treated lightly with sulfuric acid (4 per cent sulfuric acid for ten minutes, followed by neutralization with thirty per cent sodium hydroxide in the presence of tincture of litmus).

Appropriate dilutions, up to 10^{-6} were prepared from the original suspension and inoculated on media with and without isoniazid. For the spleen, only medium without isoniazid was used.

Löwenstein-Jensen medium was employed (tubes of 17 mm. in diameter containing 7 ml. of medium). Isoniazid was incorporated in the medium before inspissation. The concentrations of isoniazid added per ml. of medium were: 0.05 γ; 0.1 γ; 0.2 γ; 0.5 γ; 1 γ; 2 γ; 5 γ; 25 γ; and 100 γ. Inspissation lasted fifty minutes at 85° C. Two tubes of medium without isoniazid and two tubes of each concentration of isoniazid, 20 tubes in all, were inoculated with each of the chosen dilutions at the rate of 0.2 ml. per tube. The cultures were examined on the twenty-fifth and fiftieth days, and the colonies were counted whenever possible. The highest count was adopted. In the very rare cases in which the undiluted suspension gave an average number of colonies below 50 on the control medium, the study of the composition of the population was repeated by indirect titration after passing the primary culture through Dubos liquid medium without isoniazid.

Measure of active isoniazid concentration in blood: The measure was made on mice of the same breed, but different from those used in the infection experiments. For each dose of isoniazid, one batch of 20 mice was employed. The animals received 0.1 mg. or 1 mg. of isoniazid daily during three days. Two hours after the dose on the third day the animals were bled and the blood of all of the animals of each treatment group was pooled. The concentration of active isoniazid in the serum was measured by a biologic method. Because of the small amount of serum available, the method employed was not that of vertical diffusion usual in this laboratory (11, 12), but the method in liquid medium previously described (13).

Each test was repeated. The estimate of the amount of active isoniazid in the serum was then repeated with each dose of isoniazid on a different batch of mice.

RESULTS

Only the populations of the lungs will be considered here. The detailed results of the experiments are shown in table 2. The numbers of bacilli in this table always relate to the whole lung. In column No. 1 the total bacillary population for each lung is shown, calculated by multiplying by the dilution factor the average number of colonies obtained per tube of medium without isoniazid. Next are shown the different fractions, susceptible and resistant, of this population. Six fractions were considered: *susceptible* bacilli with resistance below 0.05 γ per ml. (column No. 2); bacilli with resistance equal to or above 0.05 γ per ml., but below 0.1 γ per ml. (column No. 3); bacilli with resistance equal to or above 0.1 γ per ml. but below 1 γ per ml. (column No. 4); bacilli with resistance equal to or above 1 γ per ml., but below 25 γ per ml. (column No. 5); and bacilli with resistance equal to or above 25 γ per ml. (column No. 6).

Each of these fractions was calculated by deducting the average number of colonies on the tubes of higher concentration of isoniazid from the average number of colonies on the tubes of lower concentration of isoniazid. For example, the fraction of column No. 3 was obtained by deducting the average number of colonies on the tubes with 0.1 γ per ml. from the average number on the tubes with 0.05 γ per ml. Thus, the figure in column No. 3 for mouse No. 8 signifies that there were in the entire lung of this animal 15,000 bacilli the resistance of which was equal to or above 0.05 γ per ml., but below 0.1 γ per ml. of isoniazid.

The populations were calculated whenever possible, starting from dilutions which gave from 10 to 100 colonies per tube. The population figures below 500 have only an approximate value, as they correspond to less than 10 colonies per tube: they simply indicate an order of size. The fractions of the populations were also expressed in percentages. These percentages refer to the total population of the lung. The last two fractions

were put together for calculating the percentages (column No. 7).

Each strain was classed as susceptible or resistant (column No. 8) according to the following definition: a strain containing at least 1 per cent of bacilli resistant to 0.1 γ or more of isoniazid per ml. was called resistant. This extremely low level of resistance was chosen deliberately in order to stress even small deviations from the state of full drug susceptibility. (In wild strains, the proportion of variants resistant to 0.1 γ of isoniazid per ml. lies between 0.01 and 0.0001 per cent.) The evolution of the populations will first be described separately for each treatment group, and the two evolutions will then be compared.

Initial Composition of the Bacillary Populations

The initial composition of the two strains inoculated, as well as the composition of the strain H37Rv, has been indicated in table 1. The measurements were made by the same technique as the one employed for the lung populations. As stated above, strain *S.17* was slightly richer in resistant mutants than *S. 13*, which was itself richer than H37Rv. The question whether these are constant characters of the three strains cannot be considered here.

On the day 0 of treatment (the fifteenth day of the infection), the total lung populations, measured on 6 animals, ranged between 2.2×10^6 and 1.5×10^7 bacilli (median value: 7.7×10^6). Their composition, studied in 2 animals, was approximately the same as that found *in vitro* for the two strains used. This was better shown by study of 2 *nontreated* animals, sacrificed thirty-four days later (forty-ninth day of the infection), as the total populations were by then higher (5×10^7 and 2×10^7 bacilli), which reduces the error of estimating the rarest mutants. The composition of these populations stayed about the same as that of the inoculated strains, which indicates that the speed of multiplication has not been radically different for the different mutants. However, slightly slower multiplication of mutants of high resistance is probable.

Changes of Populations in the Group Treated with 0.1 Mg. of Isoniazid

On the fourteenth day of treatment, the total population had not diminished at all (median value 1.1×10^7, against 7.7×10^6 at day 0). No fall was recorded for either the susceptible or the resistant fractions of the population.

On the thirty-fifth day, the total population had begun to fall (median value 4×10^6); only one animal had a population greater than 10^7, compared with 3 animals treated previously. This fall occurred only in the susceptible population; the resistant fraction at 0.05 γ per ml. remained practically unchanged.

At the seventieth day, the fall of the total population had continued (median value 8×10^5), but it was not now confined only to the susceptible fraction; *the fraction resistant at 0.05 γ per ml. had also dropped.* This fraction had totally disappeared in 4 animals and was considerably diminished in 2 others. The populations of higher resistance were still present. Taken as a whole, the strains remained susceptible.

At the one hundred and fifth day, the total population had again dropped (median value 2×10^5); in 2 animals (Nos. 45 and 46) only very small residual populations of the order of 10^3 bacilli were found. As before, this fall concerned first the susceptible fraction of the populations; but now the fraction resistant at 0.05 γ per ml. had disappeared in all of the animals, and another fraction, the fraction resistant at 0.1 γ per ml., constantly present up to now, had also disappeared. Taken as a whole, the strains of 4 animals remained susceptible. The fraction with a resistance equal to or greater than 1 γ per ml. (column No. 7, table 2) varied considerably from one animal to another. In 2 animals, it was completely absent (Nos. 45 and 46); these animals had only a small population, entirely susceptible. Conversely, in 2 animals (Nos. 43 and 44), it had become fairly large, attaining a figure of 3×10^4 and 8×10^5 bacilli; in this latter case, the bacillary population consisted entirely of the resistant fraction. The *absolute* increase of highly resistant populations in several animals is a fact of great importance.

At the one hundred and fortieth day, the total populations had become fairly small, of the order of 10^3 and 10^4 bacilli. The dominant feature was the marked difference between animals. In one animal (No. 53), the whole population was entirely susceptible; in another (No. 51) it was more than 80 per cent susceptible. Conversely, in 2 animals (Nos. 52 and 54), only highly resistant populations were found. Admittedly, the popula-

TABLE 2

TOTAL POPULATIONS OF MYCOBACTERIUM TUBERCULOSIS, SUBDIVIDED INTO SEVERAL FRACTIONS ACCORDING TO THEIR DEGREE OF RESISTANCE TO ISONIAZID, ISOLATED FROM MOUSE LUNGS AT DIFFERENT INTERVALS DURING A TREATMENT BY 0.1 MG. OR 1 MG. OF ISONIAZID

Treatment Started 15 Days after Infection

Day of Treatment	Isoniazid Dosage	Animal Number	Total Population		Susceptible Fraction <0.05 γ/ml		Resistant Fraction 0.05 – <0.1 γ per ml		Resistant Fraction 0.1 – <1 γ per ml		Resistant Fraction 1 – >25 γ/ml			Qualification of the Strain
			Number*	Per Cent	Number*	Per Cent	Number*	Per Cent	Number*	Per Cent	1 – <25 Number*	≥25 Number*	1 – >25 Per Cent	
		Column	1		2		3		4		5	6	7	8
	mg.													
0	0	Median Value†	7.7×10^6		7.6×10^6		7.4×10^4		9.7×10^2		1.1×10^2	2.1×10^1		S‡
14	0.1	5	1.1×10^7	±100	1×10^7	95.5	5×10^5	4.5	4×10^3	0.04	5×10^1	0	0.0005	S
		6	1×10^7	±100	9.5×10^6	95	5×10^5	5	1×10^3	0.01	5×10^1	0	0.0005	S
		7	1.1×10^7	±100	1.1×10^7	98.2	2×10^5	1.8	2×10^2	0.002	5×10^2	5×10^1	0.005	S
		8	9.5×10^5	±100	9.3×10^5	98.4	1.5×10^4	1.6	5×10^1	0.005	0	0	0	S
		9	1.7×10^6	±100	1.7×10^6	99.6	6.7×10^3	0.4	2×10^2	0.01	4×10^1	0	0.002	S
		10	2.1×10^7	±100	2×10^7	97.6	5×10^5	2.4	9×10^2	0.004	4×10^2	3.5×10^2	0.004	S
		Median Value	1.1×10^7		9.7×10^6		3.5×10^5		5.5×10^2		5×10^1	0		
	1	11	1.3×10^6	±100	1.3×10^6	97.4	3.4×10^4	2.6	5×10^2	0.04	0	1×10^1	0.0008	S
		12	2×10^6	±100	2×10^6	99.4	1.2×10^4	0.6	0.8×10^2	0.004	7×10^1	5×10^1	0.004	S
		13	1.5×10^5	±100	1.5×10^5	98.5	2.3×10^3	1.5	2×10^2	0.1	0	0	0	S
		14	7×10^5	±100	6.9×10^5	99	6.8×10^3	1	1.5×10^2	0.02	0	5×10^1	0.007	S
		15	9×10^5	±100	9×10^5	99.8	1.5×10^3	0.2	1×10^1	0.001	0	0	0	S
		16	1.6×10^6	±100	1.6×10^6	99.8	3.4×10^3	0.2	5.5×10^1	0.003	0	2×10^1	0.001	S
		Median Value	9.7×10^5		9.6×10^5		5.1×10^3		1.1×10^2		0	1.5×10^1		

		17	1.9×10^6	±100	1.8×10^6	94	1.1×10^5	6	6×10^2	0.03	1×10^1	0	0.0005	S
		18	6×10^6	±100	5.4×10^6	90	6×10^5	10	8.5×10^2	0.014	0	1.5×10^2	0.002	S
		19	1.5×10^7	±100	1.4×10^7	99	1.5×10^5	1	1×10^3	0.007	0	1×10^3	0.007	S
	0.1	20	9×10^5	±100	8.7×10^5	97	2.3×10^4	3	1×10^3	0.1	0	1×10^3	0.1	S
		21	1.1×10^6	±100	Infection		Infection		Infection		Infection	1.5×10^2		
		27	6×10^6	±100	5.6×10^6	94	3.5×10^3	6	2×10^2	0.003	0	0	0	S
		Median Value	4×10^6		(5.4×10^6)		1.1×10^5		8.5×10^2		0	1.5×10^2		
35														
		23	3×10^6	±100	3×10^6	99	2.7×10^4	0.9	0	0	0	2.5×10^3	0.08	S
		24	1.5×10^5	±100	1.3×10^5	90	0	0	8.5×10^3	6	4×10^2	1.5×10^3	4	R§
		25	6.5×10^4	±100	6.4×10^4	99	5×10^2	0.8	0	0	0	0	0	S
	1	26	1.8×10^5	±100	1.1×10^5	64	1.5×10^4	8	0	0	0	5×10^4	28	R
		27	1×10^5	±100	7.2×10^4	72	0	0	0	0	0	2.8×10^4	28	R
		28	1×10^4	±100	5.5×10^3	55	0	0	0	0	3.6×10^3	9×10^2	45	R
		Median Value	1.3×10^5		8.6×10^4		2.5×10^2		0		0	2×10^3		
		29	3×10^5	±100	2.9×10^5	98	5×10^3	1.7	0	0	0	1×10^1	0.003	S
		30	1.2×10^6	±100	1.2×10^6	99	9.3×10^3	0.8	6.5×10^2	0.05	0	0	0	S
		31	1×10^6	±100	1×10^6	99.9	0	0	1×10^3	0.1	1×10^2	1×10^2	0.02	S
	0.1	32	5×10^5	±100	5×10^5	99.9	0	0	3×10^2	0.06	1.7×10^2	2.5×10^1	0.04	S
		33	5×10^5	±100	5×10^5	99.6	0	0	2×10^3	0.4	2×10^2	5×10^1	0.05	S
		34	2.5×10^6	±100	2.5×10^6	99.6	0	0	8.8×10^3	0.4	0.7×10^2	5×10^2	0.02	S
		Median Value	8×10^5		7.5×10^5		0		8.2×10^2		0.8×10^2	3.7×10^1		
70														
		35	0		0		0		0		0	0		
		36	1.5×10^4	±100	1.5×10^4	99.7	0	0	0	0	0	5×10^1	0.3	S
		37	5×10^2	±100	0	0	0	0	0	0	0	5×10^2	100	R
	1	38	1×10^3	±100	0	0	0	0	0	0	7×10^2	3×10^2	100	R
		39	1×10^4	±100	0	0	0	0	0	0	0	1×10^4	100	R
		40	2×10^2	±100	0	0	0	0	0	0	0	2×10^2	100	R
		Median Value	8×10^2		0		0		0		0	2.5×10^2		

TABLE 2—*Continued*

Day of Treatment	Isoniazid Dosage	Animal Number	Total Population		Susceptible Fraction <0.05 γ/ml		Resistant Fraction 0.05 – <0.1 γ per ml		Resistant Fraction 0.1 – <1 γ per ml		Resistant Fraction 1 – >25 γ/ml			Qualification of the Strain
			Number*	Per Cent	Number*	Per Cent	Number*	Per Cent	Number*	Per Cent	1 – <25 Number*	⩾25 Number*	1 – >25 Per Cent	
		Column	1		2		3		4		5	6	7	8
	mg.													
		41	1.5×10^5	±100	1.5×10^5	98.7	0	0	0	0	0	2×10^3	1.3	R
		42	3×10^5	±100	3×10^5	99.8	0	0	0	0	0	6×10^2	0.2	S
		43	6×10^6	±100	6×10^6	99.5	0	0	0	0	0	3×10^3	0.5	S
	0.1	44	8×10^5	±100	0	0	0	0	0	0	0	8×10^5	100	R
		45	8×10^2	±100	8×10^2	100	0	0	0	0	0	0	0	S
		46	2×10^3	±100	2×10^3	100	0	0	0	0	0	0	0	S
105		Median Value	2.2×10^5		1.5×10^4		0		0		0	9×10^2		
		47	1×10^6	±100	0	0	0	0	0	0	0	1×10^6	100	R
		48	1×10^4	±100	8×10^3	80	0	0	5×10^2	5	3×10^2	1.2×10^3	15	R
	1	49	2.5×10^4	±100	0	0	0	0	0	0	0	2.5×10^4	100	R
		50	5×10^2	±100	Infection		Infection		Infection		Infection			
		Median Value	1.7×10^4		0		0		0		0	(2.5×10^4)		
		51	1.5×10^4	±100	1.2×10^4	83	2.5×10^3	17	0	0	0	0	0	S
		52	1.2×10^3	±100	0	0	0	0	0	0	0	1.2×10^3	100	R
	0.1	53	6×10^3	±100	6×10^3	100	0	0	0	0	0	0	0	S
		54	3×10^4	±100	0	0	0	0	0	0	0	3×10^4	100	R
140		Median Value	8.7×10^3		3×10^3		0		0		0	6×10^2		
		55	1×10^4	±100	0	0	0	0	0	0	0	1×10^4	100	R
	1	56	5×10^1	±100	0	0	0	0	0	0	0	5×10^1	100	R
		57	1×10^4	±100	0	0	0	0	7.5×10^3	75	2.4×10^3	1×10^2	25	R
		Median Value	1×10^4		0		0		0		0	3.5×10^2		

* Viable units per total lung.
† *See* text.
‡ S = susceptible (for definition, see text).
§ R = resistant (for definition, see text).

tions are very small but, in relation to the initial numbers, the increase is important.

The changes of populations which have been described can be summarized in the following way: a relatively late and slow fall of the susceptible population, which disappeared completely in only 3 animals; a still later fall of the fractions resistant to 0.05 γ per ml. and 0.1 γ per ml., both of which, however, completely disappeared in most animals; a tendency to absolute increase of the highly resistant fraction (>25 γ), which in some animals attained fairly high figures; considerable differences in the nature of populations from one animal to another; and finally, the absence of resistant strains before the one hundred and fifth day.

Changes of Populations in the Group Treated with 1 Mg. of Isoniazid

On the fourteenth day of treatment, the changes of population were already very important. The total population had clearly diminished (median value 9.7×10^5 against 7.7×10^6 at day 0); in 3 animals it was less than 10^6, while at day 0 it was not less than 10^6 in any animal. The decrease related not only to the susceptible part of the population, but equally to the fraction resistant to 0.05 γ per ml., and probably to the one resistant to 0.1 γ per ml. All strains remained susceptible.

At the thirty-fifth day, the changes had become considerable. The fall of the total population had continued (median value 1.3×10^5). It affected at first the susceptible population: In 3 animals this fraction was of the order of 10^3 to 10^4 bacilli, a figure which was not at this stage observed in any animals of the 0.1 mg. group. The fall concerned also the fractions resistant to 0.05 γ per ml. and 0.1 γ per ml. In the majority of animals they were now totally absent. On the other hand, the fraction of higher resistance (> 1 γ) clearly increased in the majority of animals. Added to the marked decrease in the susceptible population, this change conferred on the strains of 4 animals (Nos. 24, 26, 27, and 28) a character of resistance: the highly resistant fraction represents 4 to 45 per cent of the total population.

At the seventieth day, the total populations had become very small (median value 8×10^2). One animal did not even supply a single colony. The nature of the populations varied. One animal (No. 36) harbored a population almost exclusively susceptible and very much reduced. In the 4 remaining animals, *the susceptible fractions and those resistant to 0.05 γ per ml. and to 0.1 γ per ml. had entirely disappeared:* these animals harbored only highly resistant populations. In absolute value, these populations were no larger than in those animals sacrificed on the thirty-fifth day.

At the one hundred and fifth and one hundred and fortieth days, the situation remained about the same. One animal still showed a predominately susceptible population (No. 48); another (No. 57), a population in which the fraction resistant to 0.1 γ per ml. predominated. In all other animals, the populations were uniformly resistant to at least 25 γ per ml. In absolute value they remained moderate, except in one animal (No. 47), in which the population was of 10^6 bacilli, which represents a considerable increase over the initial amount.

Thus, the principal changes of populations observed in this group are the following: an early and rapid fall of the susceptible population, which disappears in the great majority of animals; an early and rapid fall of the fractions resistant to 0.05 γ per ml. and 0.1 γ per ml., which also disappear in most animals; conversely, an appreciable increase of the fraction resistant to at least 1 γ per ml., variable from one animal to another, bearing almost always upon the subfraction resistant to at least 25 γ per ml.; and finally, the presence of resistant strains from the thirty-fifth day in the great majority of animals.

Comparison between the Two Treatment Groups

The principal difference between the two treatment groups consists in an earlier fall of the populations in animals treated with the large dose of isoniazid. This fact is clearly shown by figures 1 to 5, in which the evolution of the median values of the populations in the two groups are compared.

The total population (figure 1) and the different fractions of this population (figures 2, 3, 4, and 5) are considered separately. The greater speed of fall of bacterial population in the 1 mg. group is obvious and spectacular: it relates to the total population as well as to the susceptible and weakly resistant fraction of the population (0.05 $-$ 0.1 γ per ml., 0.1 $-$ 1 γ per ml.). However, after the seventieth day a slight rise of the total population occurs in the 1 mg. group; this is due

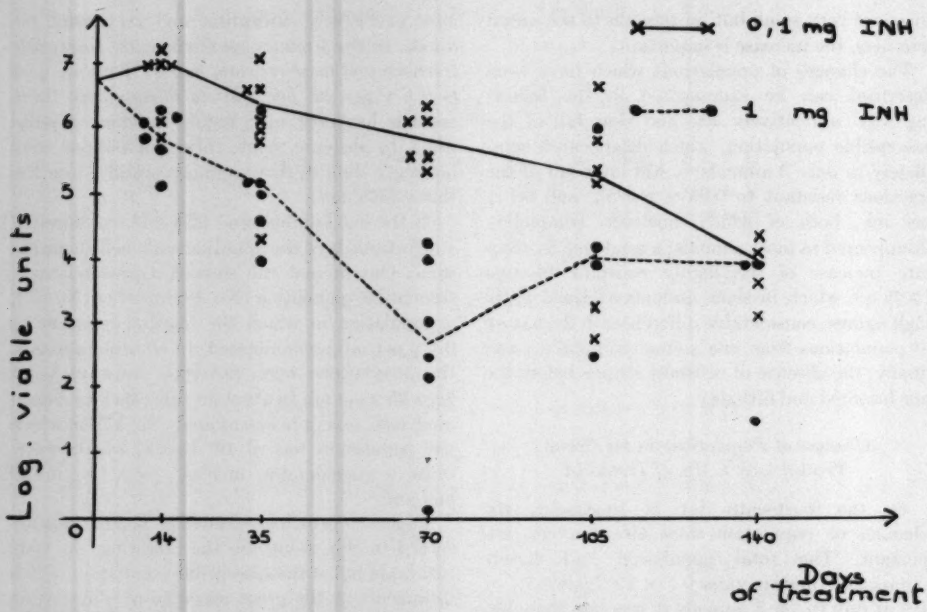

FIG. 1. Median values of populations of *Mycobacterium tuberculosis* isolated from mouse lungs at different intervals during treatment with 0.1 mg. or 1 mg. of isoniazid: *Total population.*

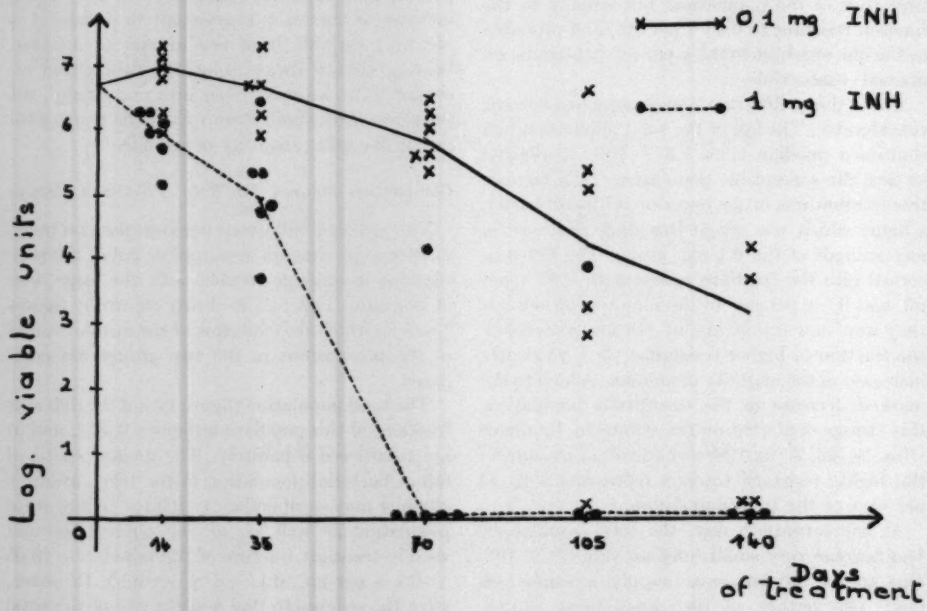

FIG. 2. Median values of populations of *Mycobacterium tuberculosis* isolated from mouse lungs at different intervals during treatment with 0.1 mg. or 1 mg. of isoniazid: *Fraction of the population susceptible to 0.05 γ of isoniazid per ml.*

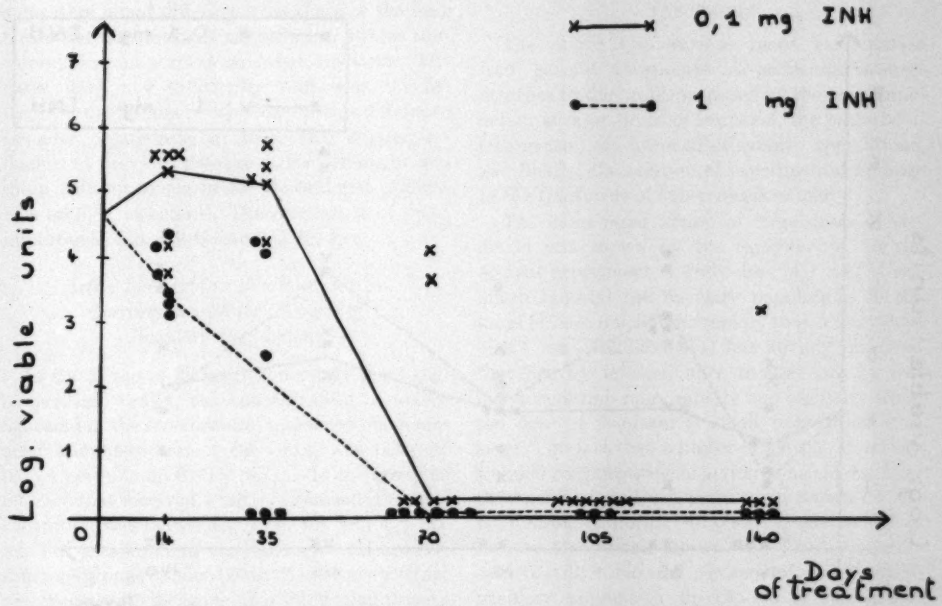

FIG. 3. Median values of populations of *Mycobacterium tuberculosis* isolated from mouse lungs at different intervals during treatment with 0.1 mg. or 1 mg. of isoniazid: *Fraction of the population resistant to at least 0.05γ of isoniazid per ml.; susceptible to 0.1γ of isoniazid per ml.*

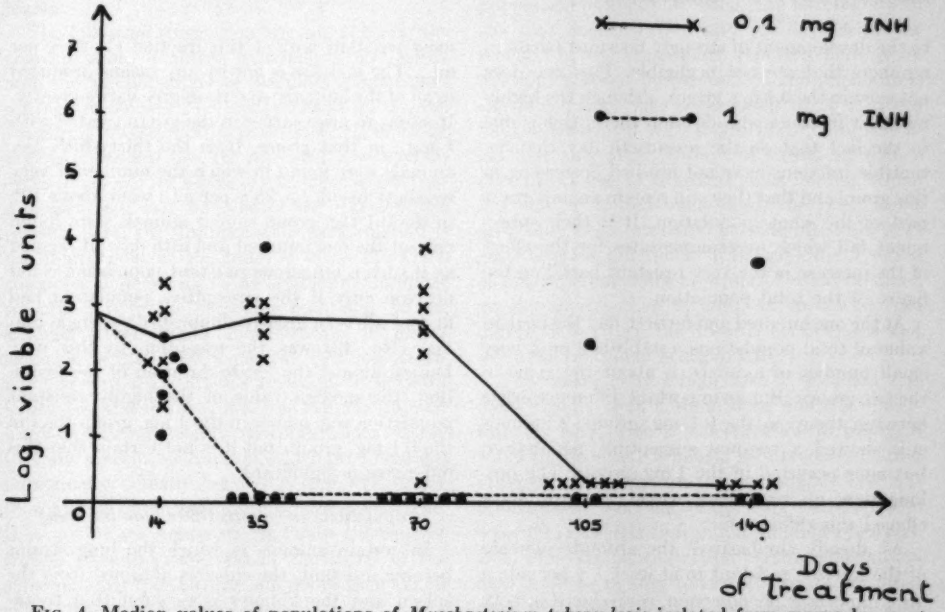

FIG. 4. Median values of populations of *Mycobacterium tuberculosis* isolated from mouse lungs at different intervals during treatment with 0.1 mg. or 1 mg. of isoniazid: *Fraction of the population resistant to at least 0.1γ of isoniazid per ml.; susceptible to 1γ of isoniazid per ml.*

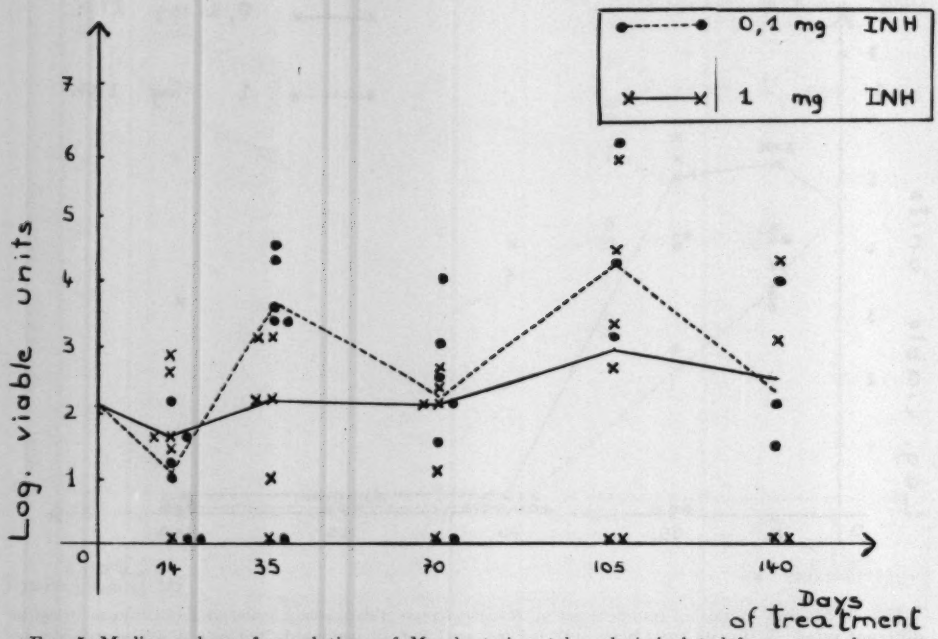

FIG. 5. Median values of populations of *Mycobacterium tuberculosis* isolated from mouse lungs at different intervals during treatment with 0.1 mg. or 1 mg. of isoniazid: *Fraction of the population resistant to at least 1 γ of isoniazid per ml.*

to the development of strongly resistant bacilli in numbers that are not negligible. This rise does not exist in the 0.1 mg. group, although the highly resistant fractions also develop there; this is due to the fact that on the seventieth day the susceptible fractions have not finished decreasing in this group and that they still remain an important part of the whole population. It is their subsequent fall which overcompensates for the effect of the increase in the very resistant bacilli on the figure of the total population.

At the one hundred and fortieth day the median value of total populations, established on a very small number of animals, is about the same in the two groups. But an important difference exists between them: in the 0.1 mg. group, 2 animals still showed a residual susceptible population; but none occurred in the 1 mg. group. The prolongation of treatment would perhaps have effaced this difference.

As already emphasized, the absolute increase of the fraction resistant to at least 1 γ per ml., a very important phenomenon, is produced in both treatment groups. Actually, it relates only to the most resistant part of this fraction (> 25 γ per ml.). The increase is not by any means produced in all of the animals; and its degree varies greatly. It seems to arise earlier in the group treated with 1 mg.; in that group, from the thirty-fifth day animals were found in which the number of very resistant bacilli (> 25 γ per ml.) went above 10^4; in the 0.1 mg. group similar animals were found only at the one hundred and fifth day. It seemed as if such a numerous resistant population could develop only if the susceptible population had first of all very greatly diminished: a single animal (No. 43) was the exception to this rule. During almost the entire duration of the evolution, the median value of the highly resistant population was higher in the 1 mg. group than in the 0.1 mg. group; but it is not certain that this difference is significant.

Populations in Organs Other than the Lung

In certain animals in which the lung strains became resistant, the cultures obtained from the spleen and the kidneys were submitted to *indirect* population studies. In some cases the re-

sults were found different from those of the lung strains: strains totally susceptible, or strains containing less important resistant fractions. The cases were not sufficiently numerous to distinguish the factors responsible for this difference (smaller populations in these two organs; influence of previous passage of the strains in medium without isoniazid for the indirect population studies, et cetera). The problem is of great importance and will be studied further.

Active Isoniazid in the Blood Serum. Correlation with the Changes of Bacillary Populations

In the group of 20 mice that received 0.1 mg. of isoniazid orally, the concentration of active isoniazid in the blood serum, measured two hours after administration of the drug, was between 0.25 γ per ml. and 0.50 γ per ml. In the group of 20 mice that received 1 mg. of isoniazid, the concentration was between 2 γ per ml. and 4 γ per ml. The measurement was done a second time on different groups of mice (20 to 25 mice per group); the results were the same. It is interesting to note that a dose ten times as strong has produced, in the conditions of the experiment, a concentration of active isoniazid in the serum nearly ten times higher.

The following correlation appears between the concentration of active isoniazid in the serum and the modifications of bacillary populations during treatment. *The disappearance of a given fraction of the population is the more rapid as the difference between the free isoniazid concentration of the serum and the degree of resistance of the fraction increases.* But the size of the fraction also plays a part; the disappearance of a fraction is the more rapid as the number of bacilli which composes it decreases. This factor predominates sometimes over the preceding one; thus, in the animals whose blood concentration of active isoniazid is 0.25–0.50 γ per ml. (0.1 mg. treatment group) the fractions resistant to 0.05–0.1 γ per ml, and even to 0.1–1 γ per ml., numerically of little importance, disappear more rapidly than the susceptible fraction, which predominates numerically. Finally, save for rare exceptions, there are no significant absolute increases of fractions with a resistance clearly below the concentration of active isoniazid in the blood of the corresponding treatment group (third hour). This will be discussed subsequently.

Discussion

The above observations merit examination from several viewpoints. A particular interest attaches to the problems posed by the more rapid action of large doses of isoniazid, the patterns of emergence of isoniazid-resistant populations, and finally, the relation of experimental findings to the treatment of tuberculosis in man.

The more rapid action of large doses of isoniazid was shown by the experiments. In the present experiment, a daily dose of 1 mg. of isoniazid reduced the bacillary populations in the lungs of mice much more rapidly than a daily dose of 0.1 mg. Noufflard (14) has already observed that heavily infected mice treated late by isoniazid succumb more rapidly and regularly when the dose of isoniazid is small than when it is large. The fact that a higher dose, that is to say a higher concentration of active isoniazid reaching the bacilli, exercises a more rapid action on the population conforms to observations made *in vitro* by Hobby and Lenert (15). The time necessary to kill *in vitro* 99 per cent of a susceptible bacillary population (in process of multiplication) is shorter when the surrounding concentration of isoniazid is 10 γ per ml. than when it is 1 γ per ml.; the times indicated by the writers for a population of about 10^5 bacilli are four days with the first concentration and six days with the second. In the experiment reported here, a reduction of about 90 per cent of the populations (median value) has been found after fourteen days of treatment with a dose of 1 mg. of isoniazid, but only after 70 days of treatment with a dose of 0.1 mg. of isoniazid. This finding, which has only a limited value since the populations have only been estimated at wide intervals, suggests that the differences of the rapidity of action of two different doses of isoniazid could be much greater *in vivo* than *in vitro*, at least when the difference between the doses is considerable and when the treatment is begun late.

The reasons why a high dose of isoniazid acts more rapidly than a small dose are still uncertain. *In vitro*, with a concentration of isoniazid equal to the active isoniazid serum concentration obtained in the mouse two hours after oral administration of 0.1 mg. of the drug, i.e., 0.25–0.50 γ per ml., a bactericidal action would be observed. But, while the concentration remains constant *in vitro*, it drops rapidly *in vivo*. It may be surmised that, with the indicated dose, the situation with

respect to the amount of active isoniazid available in the blood serum *through the twenty-four hours* is such that the action of the drug tends to become bacteriostatic rather than bactericidal. To resolve this problem in a precise manner, an experimental model reproducing *in vitro* the fluctuations of the active isoniazid concentration occurring *in vivo* would be very desirable. If the hypothesis formulated here were confirmed by appropriate experiments, the facts observed in the mice would be easy to understand. The proportion of bacilli destroyed by the direct bactericidal action of the drug, and not by an action of the defensive mechanisms of the host on bacilli simply inhibited in their multiplication, would be much higher in animals treated by 1 mg. of isoniazid than in animals treated by 0.1 mg., whence the quicker decrease of populations in the former animals.

The same hypothesis would also explain in a satisfactory manner why, even in the animals treated by 1 mg., the fractions weakly resistant, although numerically small, commence to fall later than the susceptible population. With regard to these fractions, the active isoniazid concentration produced in the serum by a 1-mg. oral dose would be too small for the action of the drug to be predominantly bactericidal.

Differences in receptivity of the bacilli to the action of the drug, according to their metabolic state, have also great importance for the interpretation of the facts observed in this experiment. McDermott (16) has long drawn attention to this factor, which intervenes in many infections: the reactions of the host tend to create a medium in which the bacterial metabolism becomes less and less active. Joined to fluctuations in the amount of active isoniazid, this most probably explains why, whatever dose of isoniazid is administered, *the fall of bacillary populations is much slower in vivo than in vitro*. This factor probably exerts a greater influence when the infection is treated in a later stage, that is to say, nearer the moment when the multiplication of bacteria ceases spontaneously. When the treatment is begun at the same time as the infection, the fall of populations is earlier than in our present experience (17).

With a feeble dose of isoniazid, residual susceptible populations persist in certain animals after one hundred and forty days of treatment. This fact is consistent with the observations made by McCune, Tompsett, and McDermott (17–19), and by Grumbach (1, 2) during prophylactic treatment with isoniazid either alone or in various combinations. In certain cases, as those writers have shown (isoniazid–pyrazinamide), the persistent bacilli cease to be cultivable, then become cultivable again after a fairly long delay of apparent sterility of the organs. These observations have led to a rather pessimistic view as to the possibilities of *eradication* of a heavy tuberculous infection by chemotherapy. It will be noticed that, in all of these studies, the treatment did not last more than one hundred and fifty days. In man, the studies of Canetti and Grosset (20) on isoniazid-resistant bacillary populations of cavities surgically resected have shown that after a year of isoniazid therapy these populations contain in only 2 per cent of positive cases a fraction of susceptible bacilli (7 of 215 cases). After two years of treatment bacilli susceptible to isoniazid were found in none of 102 cavities from which tubercle bacilli were cultured. However, the mechanisms by which isoniazid makes the last susceptible bacilli disappear from an isoniazid-resistant population are perhaps not the same as those by which this drug sterilizes an infection.

With respect to the patterns of emergence of isoniazid-resistant populations, there is reason to examine first why the development of isoniazid-resistant populations occurred so frequently in the animals of this study. During prophylactic treatment (treatment begun at the same time as the infection), McCune and Tompsett (17) have only exceptionally observed such populations. The two strains used in this experiment may lend themselves more readily to the development of resistant populations than strain H37Rv used by McCune and Tompsett. But the main reason for the difference probably lies elsewhere. During treatments which began late, as in the present experience, Grumbach has very often obtained resistant populations in mice infected *with the H37Rv strain* by employing various drugs alone: isoniazid (1), streptomycin (1), pyrazinamide (2), ethioniamide (13). Therefore, in all likelihood the delay in treatment is the determining factor. The mechanism by which a treatment commenced late favors the appearance of resistant populations is still uncertain. The most plausible factor is the considerable increase in the bacillary population in the interval without treatment: the absolute number of resistant mutants in the organism being larger, the chances are much greater that some of the mutants selected during treatment dwell in conditions sufficiently favorable for

active multiplication to take place. If this hypothesis is correct, the use of a sufficiently large inoculum should permit frequent development of resistant populations even when treatment is prophylactic. This has been observed in the rat (21) and is being studied in the mouse. Nevertheless, the possibility that factors other than the initial number of bacilli are concerned cannot be excluded.

The levels of drug resistance that were observed were of considerable interest. Except for rare exceptions, the resistant populations which existed in animals after one hundred and five and one hundred and forty days of treatment (and even after seventy days in the animals treated by 1 mg.) comprised only bacilli of a very high resistance ($> 25\ \gamma$ per ml.).

The resistance level of mutants which multiply during treatment by isoniazid depends on two factors: the concentration of active isoniazid in the blood and the initial structure of the strain. As far as the first factor is concerned, Canetti and Grosset (9) have shown, in cases of human pulmonary tuberculosis resected after *prolonged* isoniazid therapy, that the concentration of active isoniazid present in the blood serum three hours after administration of a dose of isoniazid (or isoniazid-PAS) equal to that absorbed at each intake during treatment represents a kind of barrage as far as the multiplication of resistant mutants is concerned. If the lesions contain an abundant population, that is to say, if the bacilli did multiply during treatment, the concentration of isoniazid to which 100 per cent of the population are resistant is almost always above the blood concentration of active isoniazid present at the third hour. It happens, however, that bacilli of a resistance below the therapeutic isoniazid concentration are found in the resected lesions. But, although this eventuality is rare (12 of 94 cases), only very small numbers of bacilli are found in such cases, that is to say, according to all probability, a pre-existing population which did not multiply during treatment. This situation was verified in the present experiment: save perhaps in one animal (No. 57), there was no multiplication of bacillary fractions in which the resistance was definitely below the concentration of active isoniazid in the blood of the corresponding treatment group.

It may be wondered, however, why in the animals treated by 0.1 mg. of isoniazid the resistant populations finally present have always been of a fairly high level of resistance. With a concentration of active isoniazid of 0.25–0.50 γ per ml., it would have been expected that, in some animals at least, the level of resistance of the population would have been less: of the order of 1 γ per ml., for example. In the work cited above, in 17 cases of human tuberculosis in which the amount of active isoniazid in the blood during treatment (third hour) was less than 0.4 γ per ml., that is to say, comparable to that of mice treated by 0.1 mg. of isoniazid, 10 patients showed a bacillary population of a resistance higher than 1 γ per ml.; but 7 patients, a population of a resistance somewhat below 1 γ per ml. The difference found in the present experience cannot be explained with certainty. It is probably the initial structure of the strains which is important. In wild strains the distribution of mutants of different resistance shows a common pattern; but fairly large variations of detail do occur, a fact also emphasized by Middlebrook (22). For example, in many wild strains, the number of mutants giving colonies on 0.2 γ per ml. of isoniazid is considerably higher than that giving colonies on 25 γ per ml.; in other strains, the difference is fairly small. A more important difference probably rests in the fact that, in certain strains of the first type, many mutants which multiply on 0.2 γ per ml. are only phenotypically of low resistance; their descendants adapt themselves very quickly, sometimes from the first passage, to high concentrations of isoniazid, which demonstrates that the variants involved are genotypically different. These differences explain why the level of resistance of populations selected by the same concentration of active isoniazid can vary according to the strain present. This factor of variation has much less chance to intervene in this experiment, in which only 2 strains were employed, than in the 17 cases of human tuberculosis mentioned above, in which different strains must have been present.

The facts and hypotheses presented so far can offer some explanation of the frequency of isoniazid-resistant populations during the present experience, as well as the level of resistance attained. But they allow a fundamental obscurity to remain: the problem of *how the selected resistant mutants could multiply so well in certain animals*. This multiplication is not brought about by itself; it is, when all is said and done, a very

abnormal phenomenon, which cannot be explained without taking into consideration some supplementary mechanisms which are still obscure.

Sever and Youmans (23) have shown that the multiplication of the bacilli in the lungs of mice inoculated with a moderate number of virulent bacilli spontaneously stops toward the fourteenth day; the population attained remains at this level. For example, in the experience of those writers, using an intravenous inoculum of the order of 4×10^4 bacilli (strain H37Rv), which produces at the second day a population in the lung of about 6×10^3 bacilli, the maximal lung population reached towards the fourteenth day does not pass 10^5 bacilli, and henceforth remains unchanged. As those writers emphasize, this stoppage of multiplication is probably due to the development of acquired immunity.

In the light of this situation, three factors appear which should specially limit the development of resistant populations in the course of isoniazid therapy: the mean number of resistant mutants initially present, the attenuation of their virulence, and the high degree of immunity produced by the susceptible bacilli of the inoculum. In the present experiment the number of highly resistant mutants present in the entire lung at the onset of treatment was below 10^2, that is to say 100 times less than in the experiment cited above: at best, the population finally attained should not have passed an amount of the order of 10^3 bacilli. Moreover, the virulence of isoniazid-resistant mutants is much diminished: strains of little virulence multiply in the lung of the mouse much less actively than do virulent strains (24). Finally, with the very large dose of susceptible bacilli in the inoculum, specific immunity had without doubt developed early. In the mouse (25, 26) as in the guinea pig (27–29), even *immediate* treatment by isoniazid does not prevent the appearance of specific immunity if the inoculum is large; moreover, in the present experiment, treatment was not immediate. These three factors acting together should have strongly opposed the multiplication of resistant mutants. Examination of the populations yielded in the present experiment shows that this was so in a great number of animals, *but not in all*. In 40 animals treated during at least thirty-five days, 9 have shown a resistant population ($> 25\ \gamma$ per ml.) equal to or greater than 10^4 bacilli; in 2 cases, the population was even about 10^6 bacilli. Without doubt, the populations in these 9 animals were very much larger than might have been expected.

The factors responsible for this situation are still obscure. What principally distinguishes the multiplication of the rare resistant mutants in the present experiment from the multiplication of a small inoculum in an experiment of the type of that of Sever and Youmans is that the first is produced in the presence of isoniazid. It is tempting to invoke a *stimulating* action of the drug on the isoniazid-resistant mutants. This action may display itself in two ways. Either isoniazid may sometimes favor the multiplication of highly resistant mutants like a growth factor; or, and more probably, its continual presence may permit numerous mutants which are phenotypically of low resistance to show themselves at the end of a certain time as being genotypically of high resistance and contribute thus to the increase of the highly resistant population. In both cases the process leading to *great* resistant populations would be partly adaptive.

Barnett, Bushby, and Mitchison (30) and Grumbach and Rist (31) have observed that mice inoculated with isoniazid-resistant strains succumb more rapidly when they receive isoniazid than when they do not. Many other facts militate in favor of adaptive processes intervening in the development of resistant populations. However, for the interpretation of the facts observed in the present experiment, an argument against the hypothesis of adaptation must be considered—the fairly small number of animals in which the populations have become abnormally high. One would have to suppose the existence of factors which would counterbalance the effects of adaptation and which would be more efficacious in certain animals than in others. *A priori*, such an hypothesis has nothing irrational.

The abnormally intensive multiplication of selected resistant mutants is seen in man as well as in mice, at least in cavitary pulmonary tuberculosis. It can even attain high degrees, since certain cavities contain a population of about 10^9 bacilli entirely isoniazid resistant. However, the fact that the process takes place in a cavity—which is not so in the mouse– confers on it two distinctive characteristics: on one hand, the possibility of the initial presence of a higher

number of resistant mutants (as the initial susceptible population of a cavity can itself attain 10^9 bacilli); and, on the other hand, less opposition by the defensive mechanisms of the host, which have every chance of being less efficacious in a cavity than in exudative lesions. Despite these differences, which render a little less incomprehensible certain high figures of resistant populations found in man, the intervention of adaptive processes here again remains probable. Formerly, this aspect of the problem of resistance in tuberculosis has not been studied extensively. The fact that the basic mechanism of resistance development is in all probability one of mutation-selection does not at all exclude that adaptive phenomena play an important role in the degree of multiplication of the selected resistant mutants. In this field, appropriate experiments based on already existing models in general microbiology are necessary.

* * *

A strong tendency has developed to administer high doses of isoniazid to man (32*a*) under the influence of the discovery of considerable individual differences in the degree of inactivation of isoniazid (33). This tendency could receive vigorous support from the experimental demonstration of the greater rapidity of action of high doses of isoniazid. But in this field, the situation is not simple and one must guard against too hasty extrapolations. The circumstances in which high doses of isoniazid could be *notably* more efficacious than the usual doses are probably fairly limited in the treatment of human tuberculosis. A brief reminder of what has already been observed in this field is not out of place.

Russell, Kass, Heaton, Dressler, and Middlebrook (32*b*) reported the results obtained by combined treatment with very high doses of isoniazid (16 mg. per kg. per day), a daily injection of 1 or 2 gm. of streptomycin, and PAS (or PAB) in patients with a low active isoniazid blood concentration. The results, in patients with fully susceptible bacilli, are among the best ever published. Only one of 153 patients had sputum positive for tubercle bacilli after eight months of treatment; and among the 104 patients followed for an average of two years after the beginning of treatment, only 2 suffered relapses. Vivien, Thibier, Grosset, and Lepeuple (34), employing a treatment regimen with a moderate dose of isoniazid (300–400 mg. a day), intravenous PAS and, in two-thirds of the patients, 1 gm. of streptomycin daily, compared the early bacteriologic and roentgenographic results, according to whether the active isoniazid blood concentration was below or above 0.5 γ per ml. (third hour). The bacteriologic results were the same in the two groups; the roentgenographic results were slightly superior in the group with the higher level. Mitchell, Riemensnider, Harsch, and Bell (35) studied the rapidity of "conversion" of the sputum obtained by high doses of isoniazid (12 mg. per kg.) and various types of associated treatments, according to whether the patients treated were slow, intermediate, or rapid inactivators of isoniazid. They noted a tendency to slower "conversion" in rapid inactivators of isoniazid. However, the differences were slight, being only a little more pronounced (although not significant) when the tuberculosis was very advanced and the accompanying treatment relatively weak.

Bernard, Jarniou, Israel, Enjalbert, and Fabre (36) compared the early bacteriologic and roentgenographic results in two groups of patients, one of which was treated by 5 mg. per kg. and the other by 15 mg. per kg. of daily isoniazid; the accompanying treatment, 15 gm. of intravenous PAS, was the same in both cases. With the higher dose of isoniazid, sputum "conversion" occurred slightly earlier, and the roentgenographic results were a little better; but none of these differences was significant.

Finally, Harris (37) studied the results obtained with four regimens, one of which consisted of 1,200 mg. of daily isoniazid and daily streptomycin, and the three others of 300 mg. of daily isoniazid and various accompanying treatments. At the end of eight months, the bacteriologic and roentgenographic results were the same with the four regimens. In considering separately the slow and rapid inactivators of isoniazid, Harris noted that, no matter what dose of isoniazid was employed, conversion occurred a little earlier in the slow inactivators than in the rapid ones. Later the differences were slight.

With some variations in detail, these studies all show the same tendency and conform to the results obtained in mice. The favorable changes are a little earlier with large doses of isoniazid than with feeble ones, in the slow inactivators than in the rapid inactivators. But in man the differences are never significant and the tendency effaces itself with time. The *final* efficacy of large doses of isoniazid studied in the first paper (32*b*) appears remarkable. But a statistical study by Crofton (38), although different in some points, provides long-term results just as remarkable

when the daily dose of isoniazid associated with other agents was only 200 mg. The associated treatment was usually strong.

Two points, among many others, distinguish the experiment reported here from studies comparing two doses of isoniazid in the treatment of man. In the present experiment, the ratio between the low dose of isoniazid and the high dose is 1 to 10; in the studies on man, it does not exceed 1 to 3 or 4. Above all, in the experiment on mice, isoniazid has been given alone; in the studies on man, it has been given with other agents. This difference is fundamental. The supplementary action exercised by the associated agents on the diminution of the bacillary population concerns not only—and necessarily—the fraction of the population weakly isoniazid resistant, but also the susceptible fraction: the more rapid drop in the total population of mice under treatment by isoniazid associated with another drug than under treatment by isoniazid alone, noted by McCune and Tompsett (17), clearly emphasizes this point. The associated treatment, then, represents *a priori* a factor of *reduction* of the margin of superiority of strong doses of isoniazid. It is very likely that this reduction is all the greater when the association employed is the best quality; the clinical evidence already available points clearly toward such a conclusion. Another effect of associated treatment, which it is not necessary to emphasize, is its action on the strongly isoniazid-resistant fraction of the population, in which frequent increase may be seen when isoniazid is given alone.

The last word on this problem has certainly not been said. But, in the present state of affairs, it is improbable that the systematic administration of isoniazid in large doses is very useful when an effective companion drug is employed. However, high doses of isoniazid should be given systematically in certain particular circumstances: in tuberculosis of exceptional severity, that is to say with exceptionally high numbers of bacilli; in tuberculosis due to strains of a slightly diminished susceptibility to isoniazid; and finally in tuberculosis of patients who are *very* rapid inactivators of isoniazid. This last indication poses a problem of the criteria of excessive inactivation adopted. It is possible that the most frequently employed criterion at the moment (blood concentration of active isoniazid less than 0.4 γ per ml., six hours after oral administration of 4 mg. per kg. of isoniazid) extends in an exaggerated manner the number of patients considered as excessive inactivators of isoniazid. The discussion of this problem, which concerns both the concentration and the timing adopted, is outside the realms of the present study.

There is, however, a situation in which the advantage of administering high doses of isoniazid asserts itself: when this drug is employed alone in the treatment of cavitary tuberculosis. It is well known that *initial* treatment of cavitary tuberculosis by isoniazid alone is undesirable. But there are in the world epidemiologic and economic circumstances in which no other treatment can be employed on a grand scale at the moment. Everything foretells that it is in these circumstances, making allowances for questions of toxicity, that the superiority of high doses of isoniazid over low doses will be most clearly seen.

Summary

This work consists of a quantitative and qualitative study of populations of tubercle bacilli isolated from the lungs of mice during delayed treatment with isoniazid. Infection was produced by two strains of isoniazid-susceptible bacilli. Treatment commenced fourteen days after infection. One-half of the animals were treated by a daily dose of 0.1 mg. of isoniazid, and the other half by a dose of 1 mg. Treatment lasted 140 days. With the small dose of isoniazid, a relatively slow and late fall of the susceptible part of the bacillary population, and a still later fall of the fractions of the populations resistant to 0.05 γ per ml. and 0.1 γ per ml., were observed. In some animals, a notable increase of the highly resistant fraction of the population ($> 25\ \gamma$ per ml.) occurred. With the large dose of isoniazid, an early and rapid fall of the susceptible population and the fractions of weakly resistant populations were observed. The increase of the highly resistant fraction occurred earlier, but was not significantly more important than in the animals treated with a small dose of isoniazid.

The evolution of the populations has also been studied in relation to the concentration of active isoniazid present in the blood serum of the animals two hours after administration of the corresponding doses of isoniazid.

Sumario

Estudios de las Poblaciones Bacilares en la Tuberculosis Experimental de los Ratones Tratados con Isoniacida

Consiste esta trabajo en un estudio cualitativo y cuantitativo de las poblaciones de bacilos tuberculosos aislados de los pulmones de ratones durante el tratamiento retardado con isoniacida. Se produjo infección con dos cepas de bacilos isoniacido-susceptibles. El tratamiento comenzó a los catorce días de la infección. A la mitad de los animales se les trató con una dosis diaria de 0.1 mgm. de isoniacida y a la otra mitad con una dosis de 1 mgm. El tratamiento duró 140 días. Con la dosis más pequeña de isoniacida, se observó una baja relativamente lenta y tardía de la fracción susceptible de la población bacilar, y una baja todavía más tardía de las fracciones de las poblaciones resistentes a 0.05 γ por ml. y 0.1 γ por ml. En algunos animales, hubo un aumento notable de la fracción sumamente resistente de la población ($>$ 25 γ por ml.). Con la dosis grande de isoniacida, se observó una baja temprana y rápida de la población susceptible y de las fracciones de poblaciones poco resistentes. El aumento de la fracción sumamente resistente ocurrió antes, pero no fué mayormente más importante que en los animales tratados con una pequeña dosis de isoniacida.

Se ha estudiado además la evolución de las poblaciones con relación a la concentración de isoniacida activa en el suero sanguíneo de los animales a las dos horas de la administración de las dosis correspondientes de isoniacida.

Resume

Etudes de populations de bacilles dans la tuberculose expérimentale de souris traitées à l'isoniazide

Ce travail consiste en une étude qualitative et quantitative de populations de bacilles tuberculeux isolés des poumons de souris pendant un traitement tardif par l'isoniazide. L'infection fut produite par deux souches de bacilles sensibles à l'isoniazide. Le traitemente commença quatorze jours après l'infection. La moitié des animaux fut traitée par des doses journalières de 0,1 mg d'isoniazide, et l'autre moitié par une dose de 1 mg. Le traitement dura 140 jours. On observa, avec la petite dose d'isoniazide, une chute relativement lente et tardive de la partie sensible de la population bacillaire, et une chute encore plus tardive des fractions de la population résistant à 0,05 γ par ml, et 0,1 γ par ml. Chez quelques animaux, il y eut une augmentation notable de la fraction fortement résistante de la population ($>$ 25 γ par ml). Avec la large dose d'isoniazide, on observa une chute rapide et précoce de la population sensible et des fractions des populations faiblement résistantes. L'augmentation de la fraction fortement résistante se produisit plus tôt, mais ne fut pas d'une importance plus grande que chez les animaux traités par une petite dose d'isoniazide.

L'évolution de la population a aussi été étudiée au point de vue de la concentration du taux d'isoniazide actif présent dans le sérum sanguin des animaux deux heures après l'administration de doses correspondantes d'isoniazide.

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THE ESTIMATION OF MAXIMAL BREATHING CAPACITY BY MEANS OF CHEST FLUOROSCOPY AND INSPIRATORY ROENTGENOGRAMS[1, 2, 3]

A Study in Patients with Pulmonary Tuberculosis

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INTRODUCTION

Fluoroscopy of the chest has been widely used by persons concerned with the evaluation of pulmonary function for clinical purposes. The senior author was first introduced to quantitative estimation of the maximal breathing capacity from chest fluoroscopy by Drs. George Wright and Roger Mitchell at Trudeau Sanatorium in 1949. In that year Wright (1) stated that fluoroscopy, although a clinical method, was in fact a laboratory exercise and should be tested for its accuracy and range of variation just as any laboratory procedure. At about the same time F. C. Warring, Jr. (2) published a detailed description of his technique for fluoroscopic evaluation of pulmonary function. Rib and diaphragmatic motion of each hemithorax was estimated, recorded separately in percentages of normal, and an estimate of differential lung function was obtained. The results of the use of this method of fluoroscopic estimate of differential lung function were also reported. In 1950, the Subcommittee on Pulmonary Function Tests of the American Trudeau Society stated (3): "A fluoroscopic examination is considered an integral part of the physical examination not only to obtain information concerning the intrathoracic lesion but also because it can furnish valuable data on lung function." The subcommittee included in its minimum requirements for evaluating a patient's pulmonary function: "a fluoroscopic examination which attempts to evaluate the magnitude, speed and distribution of pulmonary ventilation." A number of studies have been carried out in which fluoroscopy has been utilized to estimate differential lung function (4, 5) and in which the accuracy of estimate has been compared with bronchospirometric data.

Starting with the work of Hurtado and Fray in 1933 (6), roentgenograms have also been used to estimate quantitatively the lung volume compartments (7–12). Recently, sensitive photoelectric devices have been used to quantitate differences in the density of the fluoroscopic image during respiration and in different parts of the lungs (13–16). These methods have been more concerned with estimating the contribution of each lung to total function and of exploring local differences in ventilation-perfusion relationships than with estimations of maximal breathing capacity or total ventilatory function. Barden and Comroe (17) have also recently outlined and illustrated roentgenographic procedures which can yield qualitative information on pulmonary function and aid in the interpretation of the quantitative tests of lung function. The present writers know of no studies, however, in which a statistical evaluation has been made of the accuracy with which the maximal breathing capacity can be estimated from fluoroscopy of the chest. The current study was undertaken for this purpose and to compare the accuracy of estimation of maximal breathing capacity from chest fluoroscopy with an estimate made from frontal chest roentgenograms.

MATERIAL

Two groups of 100 patients were collected for analysis from the files of the pulmonary function laboratory. These were all patients with pulmonary tuberculosis who had been referred to the laboratory for evaluation of risk prior to thoracic surgery for treatment of their disease. The cases were unselected except that they represented consecutive fluoroscopic examinations by one of the writers (G. L. S.) performed on patients in *Group I* between October, 1953, and February,

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1955, and on those in *Group II* between April 1, 1957, and March 31, 1958. Thus, the two groups are separated by an interval of more than two years. In addition to fluoroscopy, all patients had inspiration and expiration frontal chest roentgenograms and at least maximal breathing capacity and vital capacity determinations.

Fluoroscopy: A standard procedure as outlined below was used during fluoroscopy. This required approximately 90 seconds of fluoroscopic time:

A brief survey was made, using full screen, to determine the general configuration of the thorax and grossly apparent disease. Using a horizontal band of almost full-screen width and approximately 8 cm. in height, the two lungs were compared from apex to base. The patient was rotated slowly on the vertical axis during this procedure.

Diaphragmatic motion was estimated during quiet and forced respirations, using the width of the patient's posterior rib interspaces as a measure of amplitude. The diaphragms were observed during sniff and cough only if some asynchrony of motion was observed.

The rate and degree of lung filling and emptying, change in pulmonary radiolucency, and respiratory mediastinal shift were observed during slow forced respiration, during maximally rapid expiration, and during rapid voluntary hyperventilation. The characteristic increase in lung density in full expiration was looked for. This was most marked in the lower third of the lungs, least marked in the upper third, and intermediate in the middle third. In assessing the rate of emptying, the time taken for a rapid complete expiration following full inspiration was noted. The time taken for this maneuver should not normally exceed four seconds.

The cardiovascular shadow was observed in the frontal and in both anterior oblique projections.

Fluoroscopic findings were recorded on a standard form (figure 1), and the maximal breathing capacity was estimated as a percentage of predicted normal. This will be referred to throughout the remainder of this paper as the fluoroscopic estimate of maximal breathing capacity.

It is difficult to set forth precisely the means by which the maximal breathing capacity was estimated from fluoroscopy. Little weight was attached to parenchymal infiltration or cavitation unless it was extensive or gave rise to obvious contraction of lung tissue or distortion of the mediastinum or lung volume. Impairment of lung filling and the degree and extent of airway obstruction, as evidenced by incomplete and delayed emptying, were considered to be of greatest importance in making the estimate of maximal breathing capacity. During the early period of learning, it was found important to fluoroscope the patient without prior knowledge of the roentgenographic or physiologic data. After the fluoroscopic estimate of maximal breathing capacity had been recorded, it was compared with the determined maximal breathing capacity. If the error of estimate was large, an attempt was made to determine the reasons for the discrepancy from a study of the roentgenograms, the detailed report of fluoroscopic examination, and occasionally by repeat fluoroscopy. Accuracy in making the estimate of maximal breathing capacity rose quite rapidly to the level present at the beginning of this study but, as will be shown below, continued to improve slowly with further experience.

Maximal breathing capacity: The maximal breathing capacity was measured, using a Robinson high velocity valve connected to a Douglas bag with 1½-inch internal diameter tubing as described by Wright (18). The valve was fitted with a face mask, or, rarely, a mouthpiece and noseclip were used. Expired air was collected for thirty seconds and measured in a Tissot gasometer, and the measurement was converted to L. per min. at 37°C., saturated with water vapor. Determinations were repeated with suitable rest between efforts until the patient appeared to have made a maximal effort, and the difference between the two highest determinations was less than 5 per cent. The largest determination was recorded. The regression formulas of Baldwin and associates (19) were used for calculating predicted normal values. Determined maximal breathing capacities were recorded as a percentage of the predicted normal.

Vital capacity: The vital capacity was determined, using a Collins 6-liter direct reading spirometer. The largest of at least three efforts was recorded. The regression formulas of Baldwin and associates (19) were used to calculate predicted normal values. The vital capacity was then expressed as a percentage of predicted, and the air velocity index (20), or ratio of maximal breathing capacity as per cent predicted to vital capacity as per cent predicted, was calculated. An air velocity index of 0.80 to 1.20 was classified as within normal limits or, when the vital capacity was less than 80 per cent of predicted, as a combined obstructive and restrictive ventilatory defect. An air velocity index of less than 0.80 was classified as an obstructive ventilatory defect, while an air velocity index of more than 1.20 in the presence of a reduced vital capacity was classified as a restrictive ventilatory defect.

Roentgenographic estimation of ventilatory function: In order to permit comparison of fluoroscopic estimation of ventilatory function with a roentgenographic estimate, the full-inspiration frontal roentgenograms taken at the time of study in the physiology laboratory were reviewed by the fluoroscopist. The review was carried out during the time of analysis of the data and when a sufficiently long time had elapsed since fluoroscopy (at least six months) that recollection of the fluoroscopic results was not possible.

A standard method of quantitating pathologic changes was employed. Each lung was divided into thirds, and the disease in each third was rated on a 4-point scale, from 0 for no disease to 3 for total destruction. Pleural involvement of the entire hemithorax was similarly rated on a 4-point scale. Thus, each lung could be assigned a maximal dis-

FLUOROSCOPY

Note: Check all abnormal findings

NAME John Hensey DATE 3-24-59 Done by: Snider

RIBS

	Right	Left
Quiet	+	+
Deep	+ + +	+ + +

DIAPHRAGM

	Right	Left
Quiet	½ ↓ p.i.*	½ ↓ p.i.*
Deep	3/4 ↑ p.i.*	1-½ ↑ p.i.*
Sniff	↓	↓
Cough	↑	↑

Synchrony Normal

Notes: Pleural scarring right base with obliteration costophrenic angle.

*p.i. = posterior interspaces

LUNGS
Gross Findings

Right	Left
Dense infiltration w/ cavitation apex. Scattered nodules below	Linear densities apex and infraclavicular
Amount and Speed of Density and Volume Change with:	
Inspiration Filling not quite complete	Fills normally
Expiration Emptics poorly with moderate delay	Emptics normally

MEDIASTINUM

Position Shifted slightly to right in upper portion on full inspiration.

Shift: + + Inspiration R ← L Expiration R → L Shift due to chron. emphysema Rt.

HEART

Chamber Enlargement 0 Notes

ESTIMATE OF FUNCTION

Right	Left
M.V. 40 O_2 25 V.C. 25	M.V. 60 O_2 75 V.C. 75
M.B.C. % Predicted 65	

FIG. 1. The form used to record fluoroscopic findings. Rib motion is recorded on a four-point scale with 4+ indicating greatest movement. Diaphragmatic excursion is estimated in posterior rib interspaces (p.i.) with a range of normal from 1½ to 2½ p.i. An estimate of differential split of minute ventilation (M.V.), oxygen uptake (O_2) and vital capacity (V.C.) is recorded as well as the fluoroscopic estimate of maximal breathing capacity (M.B.C.). The direction of diaphragmatic motion during sniff and cough and of respiratory mediastinal shift, when present, is indicated by arrows. A sketch of the parenchymal pathology is recorded on the back of the form.

ease score of 9, the pleura of 3, and a totally destroyed lung would be assigned a disease score of 12. The total disease score for both lungs and pleurae was subtracted from 24, and the difference was expressed as a percentage of 24. This percentage represented the roentgenographically estimated amount of ventilatory function. For example, in a patient with disease scores as follows: Right lung, 3; left lung, 2; right pleura, 1; left pleura, 0. Total disease score = 6. Roentgenographically estimated ventilatory function =

$$\frac{24 - 6}{24} \times 100 = 75\%.$$

RESULTS

Accuracy of fluoroscopic estimate: The difference between the fluoroscopic estimate of maximal breathing capacity and the determined maximal

TABLE 1

COMPARISON OF FLUOROSCOPIC AND ROENTGENOGRAPHIC ERROR OF ESTIMATE

Range of Error	Fluoroscopic Error* Per Cent of 200 Cases	Roentgenographic Error† Per Cent of 187 Cases
per cent		
±0–10	53	32
±10–20	26.5	33
±20–30	18	22.4
±>30	3.5	12.6

* Mean error of all cases: 11.5%
† Mean error of all cases: 17.5%

breathing capacity is the error of fluoroscopic estimate. Each case was designated as being over- or underestimated. The mean fluoroscopic error, without regard to sign, for the 200 cases was 11.5 per cent, with a range of −39 to +39 per cent and a standard deviation of 9.6 per cent (table 1 and figure 2). In 83 cases, ventilatory function was overestimated; and in 88 it was underestimated. Fluoroscopic estimate coincided exactly with determined data 29 times. Fifty-three per cent of the cases fell into the range of error of ±10 per cent. However, 21.5 per cent of the estimates were found to lie beyond the range of error of ±20 per cent.

Roentgenographic estimate of ventilatory function: Roentgenographic estimation of maximal breathing capacity was carried out on 187 patients. Films near the time of function studies were either inadequate or unavailable on 13 patients. The determined maximal breathing capacity expressed as a per cent of predicted normal was subtracted from the roentgenographic estimate, yielding an error of film estimate. The mean error of film estimate for all 187 patients without regard to sign was 17.1 per cent with a range from −54 to +35 per cent and a standard deviation of 10.5 per cent (table 1 and figure 2). This is much larger than the mean error of fluoroscopic estimate of 11.4 per cent, and the difference between the means was significant statistically ($P < 0.001$). Whereas 53 per cent of the cases fell within a range of ± 10 per cent error of fluoroscopic estimate, only 32 per cent of the cases came within the same range of error of film estimate. The error of film estimate was > 30 per cent in 12 per cent of cases, whereas for error of fluoroscopic estimate only 3 per cent of the patients were > 30 per cent. The error of fluoroscopic estimate was approximately the same in each direction, but the error of roentgenographic estimate was much more frequently in the direction of underestimating function.

The effect of experience of the fluoroscopist on accuracy of estimate: Patients in *Group I* had been studied at least twenty-four months before those in *Group II*, and in *Group I* the mean error of fluoroscopic estimate was 13.1 per cent with a range of −39 to +34 per cent and a standard deviation of 9.7 per cent. The mean

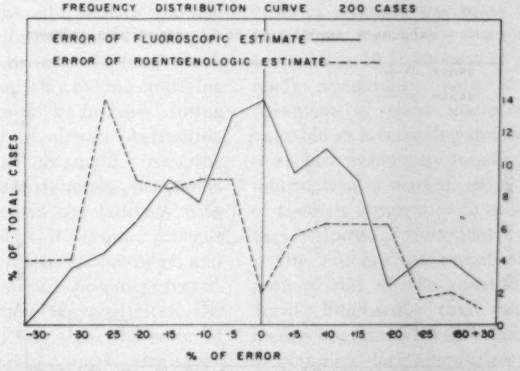
FREQUENCY DISTRIBUTION CURVE 200 CASES
ERROR OF FLUOROSCOPIC ESTIMATE
ERROR OF ROENTGENOLOGIC ESTIMATE
% OF TOTAL CASES
% OF ERROR
-30- -30 -25 -20 -15 -10 -5 0 +5 +10 +15 +20 +25 +30+30
0 2 4 6 8 10 12 14

FIG. 2. Frequency distribution curves comparing error of fluoroscopic estimate with error of roentgenographic estimate of maximal breathing capacity in 200 cases. The error of roentgenographic estimate is greater than the error of fluoroscopic estimate. The tendency is to underestimate maximal breathing capacity, using the roentgenogram, whereas fluoroscopic error is evenly distributed between over- and underestimation of maximal breathing capacity.

TABLE 2

COMPARISON OF GROUPS I AND II BY ERROR

	Group I	Group II	Difference
	per cent	*per cent*	*per cent*
Mean fluoroscopic error	13.1	9.6	3.5*
Mean error of roentgenographic estimate	18.5	15.6	2.9†

* Statistically significant: $P < 0.01$
† Statistically not significant

fluoroscopic error for *Group II* was 9.6 per cent with a range of −29 to +39 per cent and a standard deviation of 9.3 per cent (table 2 and figure 3). The difference between the means, 3.5 per cent, is significant, $P < 0.01$. The mean error of film estimate was slightly larger in *Group I* than in *Group II*, 18.5 per cent as compared with 15.5 per cent, but this difference was not statistically significant. In order to exclude the possibility that this difference between the means of error of fluoroscopic estimate was due to differences in the composition of the two groups, a comparison of the groups was made. Age, presence of mediastinal shift on respiration, previous surgery, air velocity index, amount of roentgenographically visible disease, and degree of over-all ventilatory impairment were all found to be comparable. Only sex distribution was significantly different, *Group I* containing 54 men and 46 women, and *Group II*, 69 men and 31 women. However, an analysis of fluoroscopic error by sex for the entire 200 cases showed a mean error of 11.9 per cent for men and 9.9 per cent for women. This difference is not significant; the direction of the difference also indicates that the lower fluoroscopic error in *Group II* cannot be due to the greater preponderance of men in this group. Thus, a definite effect of learning on the accuracy of the fluoroscopist is demonstrated.

Effect of mediastinal shift during respiration on accuracy of estimate: There were 57 patients in whom respiratory mediastinal shift was noted during fluoroscopy. The mean error of fluoroscopic estimate in these cases was 13.1 per cent as opposed to a mean error of 10.9 per cent in the 143 cases without shift and 11.5 per cent in all 200 cases. This difference was not statistically significant ($P < 0.06$), although it suggests that the complex phenomena observed during respiratory mediastinal motion made estimating ventilatory function from fluoroscopy more difficult. The mean error of film estimate for these 57 cases was 16.6 per cent, virtually the same as the mean of 17.3 per cent for the 143 cases without mediastinal shift and 17.1 per cent for all 200 cases. Although the accuracy of fluoroscopic estimate was not as good in the presence of respiratory mediastinal motion as in its absence, it was still significantly better statistically than the accuracy of roentgenographic estimate ($P < 0.05$).

Other factors: Age, sex, surgery prior to function

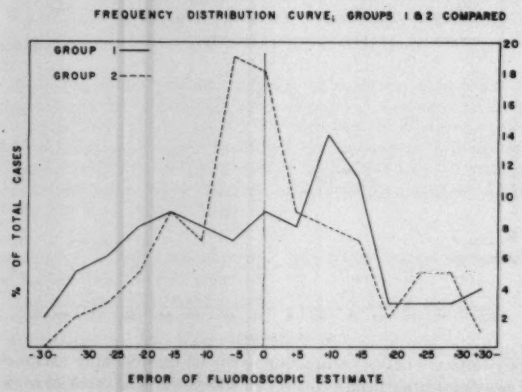

FIG. 3. Frequency distribution curves of error of fluoroscopic estimate of maximal breathing capacity in two groups of 100 cases examined by the same fluoroscopist. *Group II* cases were studied two or more years after *Group I* cases. Greater accuracy of estimation is shown in *Group II* cases.

studies, severity of disease as estimated from the roentgenographic disease score, and severity of ventilatory function impairment, as evaluated by the determined maximal breathing capacity, were found to have no significant correlation with the error of fluoroscopic estimate. There was also no significant correlation between the type of ventilatory defect as determined by the air velocity index and the error of fluoroscopic estimate.

Discussion

The significantly greater accuracy of estimation of ventilatory function from fluoroscopy of the chest than from the full-inspiration chest roentgenogram is to be expected from *a priori* reasoning. The roentgenogram gives information as to the degree of pulmonary parenchymal infiltration and destruction but gives no information regarding bronchial disease, unless the disturbance in bronchial structure and function is sufficient to produce changes in the pulmonary parenchyma. Apart from fine nodular densities which may be quite difficult to detect, fluoroscopy of the chest permits an assessment of parenchymal pathology which is superior to that obtained from the roentgenogram. In the roentgenogram, the lungs and chest wall are observed at only one point in the respiratory cycle. By rotating the patient during fluoroscopy it is possible to determine the anteroposterior location and extent of a lesion as well as its superoinferior and mediolateral extent.

Observations of volume and density changes of the lungs and of the ribs and hemidiaphragms during respiration give an index of lung ventilation. Localized or diffuse delay in lung emptying and failure of lung density to increase during expiration are indicative of airway obstruction and give information not obtainable from the roentgenogram. When these dynamic phenomena become very complex, as in the patients with respiratory mediastinal shift, it becomes difficult to assess their quantitative aspects precisely, and the error of fluoroscopic estimate becomes greater. However, even under these circumstances, the fluoroscopic error is still less than the error of roentgenographic estimate of ventilatory function. The maximal breathing capacity was underestimated from the roentgenogram in this series of patients with pulmonary tuberculosis. This occurred because the effects of parenchymal and pleural densities were given too much weight in making the roentgenographic estimate. Contrariwise, the experience of the writers is that the maximal breathing capacity is overestimated from the roentgenograms of patients suffering from idiopathic obstructive emphysema because their disease causes little change in the roentgenographic appearance of parenchyma or pleura.

Fluoroscopy of the chest has proved quite useful, especially when used in conjunction with other clinical data in screening patients prior to performing pulmonary function tests. Fluoroscopic estimates of the maximal breathing capacity have provided a helpful cross check on the laboratory tests, especially during the phase of training a technician. The accuracy of fluoroscopic estimation of maximal breathing capacity is increased as the fluoroscopist continually checks his estimate against the laboratory determination. The same is true of many other clinical tools, which are sharpened as they are used against the standard of precise study in the laboratory. An example of this is the increased accuracy of clinical diagnosis of congenital heart disease as the cardiologists have worked with cardiac catheterization and angiocardiography.

Nevertheless, even the skilled fluoroscopist is capable of gross errors, as may be seen by examining the most favorable group of patients, the 69 patients in *Group II* in whom there was no respiratory mediastinal shift. The mean error of fluoroscopic estimate of maximal breathing capacity in these patients was 8.9 per cent with a range of error from -29 to -39 per cent. The error of estimate was more than ± 10 per cent in 36.2 per cent of the cases and more than ± 20 per cent in 10.1 per cent of cases. Although the ready availability, ease of performance, and cheapness of chest fluoroscopy make it very valuable as a screening device, it cannot be used to replace pulmonary function tests when precise information is needed, as in estimating the risk of thoracic surgery or in studying the effects of various forms of treatment.

The amount of radiation exposure of the patient and of the operator is much greater during fluoroscopy than during exposure of a roentgenogram. This difference can be minimized by complete dark adaptation of the fluoroscopist, proper filtration of the X-ray beam, and the use of modern X-ray tubes and fluoroscopic screens. Total fluoroscopic time can be greatly shortened by careful training of the operator.

It is the opinion of the writers that functional

evaluation by chest fluoroscopy justifies the greater radiation exposure since it yields information not obtainable from the roentgenogram. Furthermore, it aids in the interpretation of the quantitative tests of pulmonary function and at times yields information not available from any easily applicable objective tests of lung function. Thus at times, alterations of ventilation-perfusion relationships may be inferred in a lung or even a lobe or segment when a local area of poor emptying is detected. Differential lung function can also be estimated with fairly good accuracy by means of chest fluoroscopy (21).

Summary

An analysis was carried out, using standardized procedures, of the accuracy of estimation of the maximal breathing capacity from chest fluoroscopy and from the frontal chest roentgenogram in 200 patients with pulmonary tuberculosis.

The mean error of fluoroscopic estimation for all 200 cases was 11.5 per cent with a range of −39 to +39 per cent. The error was less than ±20 per cent in 78.5 per cent of the cases. The mean error of roentgenographic estimate was 17.1 per cent with a range of −54 to +35 per cent, which is significantly larger than the error of fluoroscopic estimate. There is a decided tendency to underestimate function from the roentgenogram.

The mean error of fluoroscopic estimate for the second 100 cases (9.6 per cent) was significantly lower than that for the first 100 cases (13.1 per cent). Analysis showed the difference to be due to the development of increased skill by the fluoroscopist.

It was concluded that fluoroscopy of the chest is a cheap, readily available, and highly useful technique for screening ventilatory function. It is significantly more accurate than estimates made from frontal chest roentgenograms, but does not have sufficient accuracy to replace ventilatory function tests when precise information is needed.

Sumario

El Cómputo de la Capacidad Respiratoria Máxima por medio de la Roentgenoscopia Torácica y las Radiografías Tomadas durante la Aspiración

Este análisis, ejecutado con procedimientos normalizados, trató de determinar la exactitud del cómputo de la capacidad respiratoria máxima por la fluoroscopia torácica y por la radiografía torácica frontal en 200 tuberculosos pulmonares.

El error medio del cómputo roentgenoscópico para los 200 casos fué de 11.5 por ciento, variando de −39 a +39 por ciento. El error fué menor de ± 20 por ciento en 78.5 por ciento de los casos. El error medio del cómputo roentgenográfico fué de 17.1 por ciento, variando de −54 a +35 por ciento, lo cual es decididamente mayor que el error del cómputo fluoroscópico. Hay una tendencia indudable a menospreciar la función a la luz de la radiografía. El error medio del cómputo roentgenoscópico para los segundos 100 casos (9.6 por ciento) fué decididamente menor que para los primeros 100 (13.1 por ciento). El análisis demostró que la diferencia se debía a haber adquirido mayor destreza el fluoroscopista.

Se deduce que la roentgenoscopia del tórax es una técnica barata, accesible y sumamente útil para despistar la función ventiladora. Es decididamente más exacta que los cómputos hechos a base de las radiografías frontales del tórax, pero no posee suficiente exactitud para suplantar las pruebas de la función ventiladora cuando se necesita información precisa.

Resume

L'estimation de la capacité maxima de respiration grâce à la fluoroscopie de la poitrine et des radiographies en inspiration

Les auteurs poursuivent, en employant des méthodes ordinaires, l'analyse de la fidèlité de l'estimation de la capacité maxima de respiration par la fluoroscopie de la poitrine et les radiographies frontales de poitrine, chez 200 malades ayant une tuberculose pulmonaire.

Pour ce total de 200 cas, l'erreur moyenne de l'estimation par fluoroscopie fut de 11,5% avec une marge allant de −39 à +39%. L'erreur fut inférieure à ±20% dans 78,5% des cas. L'erreur moyenne de l'estimation par radiographie fut de 17,1% avec une marge allant de −54 à +35%, ce qui est nettement plus grand que l'erreur de l'estimation par fluoroscopie. Il y a une tendance nette à sousestimer la fonction en se basant sur les radiographies. L'erreur moyenne de l'estimation fluoroscopique pour la seconde centaine de cas (9,6%) fut nettement inférieure à celle de la première centaine (13.1%). L'analyse montra que la différence fut due au développement d'une plus grande habileté de la personne faisant la fluoroscopie.

Les auteurs concluent que la fluoroscopie de la poitrine est une technique bon marché, facile à obtenir, et très utile pour déterminer la fonction de ventilation. Cette méthode est nettement plus fidèle que les estimations faites par radiog-

raphies frontales de la poitrine, mais n'a pas assez de fidèlité pour remplacer les examens de la fonction de ventilation lorsqu'on a besoin de renseignments précis.

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SURGERY FOR PULMONARY TUBERCULOSIS IN THE PATIENT WITH LOWERED VENTILATORY FUNCTION[1]

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Introduction

Preoperative ventilatory function studies are done in the patient with pulmonary tuberculosis as one method of evaluating his ability to tolerate surgical treatment. The decision to perform surgery is not difficult in the patient with normal or slightly depressed respiratory function; however, the more the results deviate from the normal, the more difficult this decision becomes. While ventilatory studies must be used only as an adjunct to the clinical evaluation of the patient, the level of function at which no surgery should be advised and an estimate of the risk of surgery at slightly better levels would be useful.

The present attempt to relate the risk of various operative procedures to ventilatory function determinations was stimulated by a study of the causes of death in a group of patients with pulmonary tuberculosis treated by pneumonectomy. Between 1951 and 1955, 26 pneumonectomies were performed; there were 5 deaths. Each patient who died had developed a postoperative complication which compromised the function of the remaining lung. Of interest was the fact that 4 of the 5 patients had preoperative maximal breathing capacity determinations below 45 per cent of predicted normal without a comparable decrease of other functional studies (table 1). The fifth patient, who had a maximal breathing capacity of 73 per cent, died five months following the pneumonectomy during the performance of a thoracoplasty for treatment of a bronchopleural fistula and empyema. These observations suggested that any patient with pulmonary tuberculosis who had a maximal breathing capacity below 50 per cent represented a poor resectional risk, especially if pneumonectomy was contemplated. A return to the practice of pre-pneumonectomy thoracoplasty in these "poor-risk" patients was considered as a method of reducing this risk.

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Since a maximal breathing capacity at this level or below suggested an increased risk for the surgical patient, a review of the results of surgery in all patients who had maximal breathing capacities of 50 per cent or below of predicted normal was initiated.

Material and Methods

Between 1951 and 1959, 111 patients with pulmonary tuberculosis who had maximal breathing capacities below 50 per cent were treated surgically at the Ohio Tuberculosis Hospital. These patients ranged in age from sixteen to seventy-five years. One hundred of these patients had far advanced disease; 8, moderately advanced; and 3 had tuberculosilicosis (table 2).

Patients were excluded from this study if the maximal breathing capacity increased to 50 per cent or better following aerosol therapy, consisting of bronchodilators and detergents with or without positive pressure and antimicrobials topically. Bronchospirometry, residual volumes, and arterial gas studies were performed when indicated. Predictions for maximal breathing capacity were based on the age, sex, height, and weight of the subject, with due regard for the apparatus used and the method of determination of the maximal breathing capacity. A more reliable correlation of maximal breathing capacity with the postsurgical course was found if the maximal breathing capacity was expressed in percentage of deviation from a predicted normal than when this value was expressed in actual liters of air moved per minute. The innate errors of the maximal breathing capacity determinations and of expression of this value in percentile units were recognized, but minimized by multiple determinations.

One hundred and thirty-six surgical procedures were carried out in these 111 patients. These were: pneumonectomy in 13, lobectomy in 47, segmental resection in 17, therapeutic thoracoplasty in 24, extraperiosteal plombage in 12, and miscellaneous procedures consisting of decortication or closure of bronchopleural fistula in 8. Fifteen tailored thoracoplasties were done but have not been included for analysis since these were considered as a part of the total surgical approach.

Observations

In the maximal breathing capacity range between 20 and 24 per cent, 2 patients were treated successfully by collapse therapy. In the range be-

tween 25 and 29 per cent, 8 collapse procedures were done, with 2 deaths and 6 survivals (figures 1 and 2), while 3 lobectomies resulted in 2 survivals (figures 3–5) and one death. In the maximal breathing capacity range between 30 and 34 per cent, 4 patients were treated successfully by collapse procedures, 2 by lobectomies, and one by segmental resection. Three patients treated by pneumonectomy did not survive the surgical procedure. These 3 patients, however, are 3 of the 4 treated by pneumonectomy between 1951 and 1955, and did not have pre-pneumonectomy thoracoplasty. Figures 6, 7, and 8 are films of a patient who had a successful resection after thoracoplasty and whose pre-pneumonectomy maximal breathing capacity had been 35 per cent. The experience with patients who were treated by either collapse procedures or resection in the maximal breathing capacity range between 20 and 50 per cent is detailed in table 3.

The mortality rate associated with these surgical procedures in patients with low maximal breathing capacities was appreciably higher than in the patients with a maximal breathing capacity over 50 per cent. In table 4 the mortality rate of the 13 "poor-risk" patients treated by pneumonectomy is compared with that of 60 patients with maximal breathing capacities above 50 per cent, and is almost three times higher in the former group. Similarly, the mortality rate in those patients with maximal breathing capacities below 50 per cent, treated by other methods of resection or collapse, ranged between three and five times greater than in the patients with maximal breathing capacities over 50 per cent.

TABLE 1

PNEUMONECTOMY MORTALITY
1951–1955

Patients 26 Deaths 5	Number	MBC	VC	TVC	WI
		per cent	*per cent*	*per cent*	*per cent*
Causes:					
Pulmonary edema	1	34	54	—	53
Empyema	1	32	44	78	47
Spread of tuberculosis	1	35	44	87	33
Hemorrhage	1	45	56	84	23
Bronchopleural fistula and empyema*	1	73	75	95	17

* Died during subsequent thoracoplasty

MBC—Maximal breathing capacity per cent of predicted

VC—Vital capacity per cent of predicted

TVC—Timed vital capacity 3 sec. per cent of total

WI—Walking index $\frac{\text{walking ventilation}}{\text{maximal breathing capacity}}$

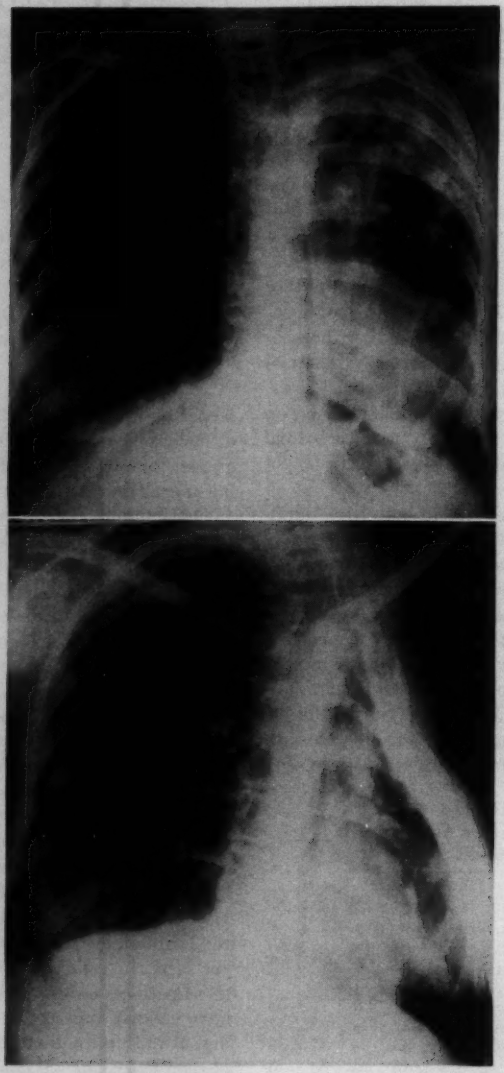
TABLE 2

SURGICAL PATIENTS, 1951–1959
Maximal Breathing Capacity 50 Per Cent or Below

Number of Patients 111	
Age range (16 to 75 years)	
16–19	2
20–29	21
30–39	28
40–49	32
50–59	22
60–69	4
70–75	2
Classification of disease:	
Far advanced pulmonary tuberculosis	100
Moderately advanced tuberculosis	8
Tuberculosilicosis	3

The complications that occurred in the patients treated by pneumonectomy and lobectomy are listed in tables 5 and 6. Comparison of the "poor-risk" patients with those with maximal breathing capacities of 50 per cent of normal or above shows a higher percentage of complications in the "poor-risk" group treated by these procedures. Also, those "poor-risk" patients who developed post-resectional complications succumbed to that complication two to three times more often than the better-risk patient.

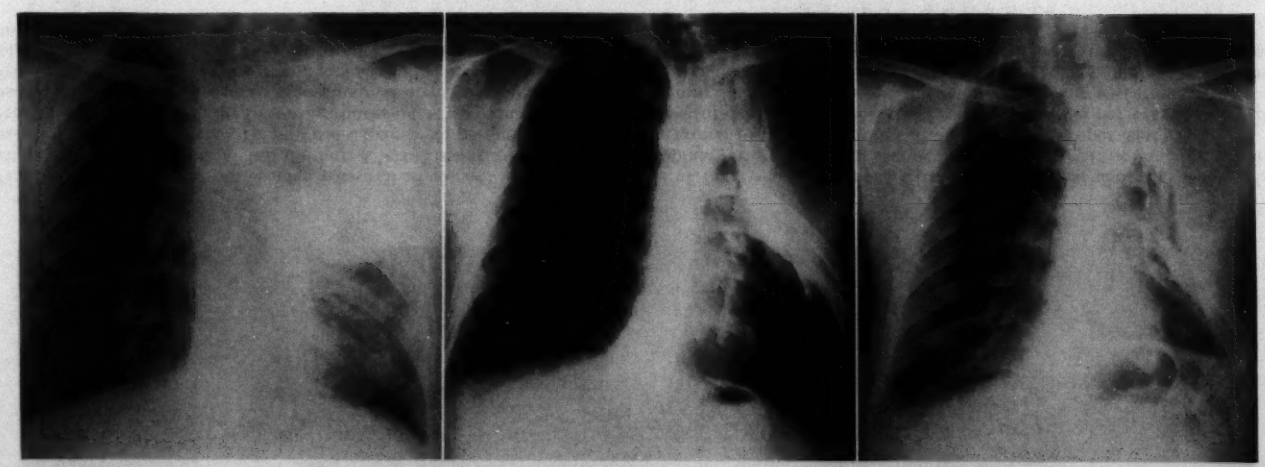
The surgical mortality rate in these 111 patients was 12.5 per cent. Additional post-convalescent deaths raised the total hospital mortality to 14.4 per cent. Post-hospitalization follow-up study has been accomplished in all but 6 of these 111 patients. Seven patients died after leaving the hospital, giving a total mortality rate of 20.7 per cent. Investigation of the present status of these patients indicates that 71 are considered to have inactive tuberculosis, 31 of whom are working, 21 full time and 10 part time (table 7). Forty of the 71 patients are not working. Four are classified by their physicians as able to work but are not doing so; 4 patients were returned to mental institutions and are still confined, although their disease is inactive; and 2 patients, one treated by lobectomy and one by thoracoplasty, have retired at the ages of seventy-nine and sixty-five years. Eleven patients still have active tuberculosis. Eight are hospitalized, 5 of whom are awaiting further surgery, 2 have refused surgery, and one



L.S.

FIG. 1. (Upper). A 30-year-old white woman admitted with far advanced, active, pulmonary tuberculosis with cavitation in the left upper lobe of five years' duration. Vital capacity 1.8 L. (57 per cent), timed vital capacity 60 per cent in 3 seconds, maximal breathing capacity 28.9 L. per min. (26.8 per cent), and walking ventilation 18.3 L. per min. A seven-rib thoracoplasty was accomplished in three stages with control of disease.

FIG. 2 (Lower). Repeat function studies eighteen months following discharge: vital capacity 1.2 L. (40 per cent), timed vital capacity 74 per cent in 3 seconds, maximal breathing capacity 17.1 L. per min. (17 per cent), and walking ventilation 14 L. per min. Patient does own housework and has successfully gone through a full-term pregnancy and delivery with minimal difficulty. She has been followed four additional years without evidence of difficulty other than "shortness of breath with exertion."



N. W.

FIG. 3 (Left). A 41-year-old man treated five years earlier with extrapleural paraffin pack. Shortly before admission, the patient had episodes of fever, dyspnea, and hemoptysis, and expectorated small pieces of paraffin.

FIG. 4. (Center). Shortly after admission the infected paraffin pack was removed and a four-rib thoracoplasty was performed. Six months later, left upper lobectomy was accomplished with excision of the bronchopleural cutaneous fistula. Preresectional pulmonary function studies: vital capacity 1.56 L. (34 per cent), timed vital capacity 96 per cent in 3 seconds, maximal breathing capacity 37.2 L. per min. (27 per cent), and walking ventilation 9.75 L. per min.

FIG. 5 (Right). Roentgenogram eighteen months following discharge from the hospital. Patient is working full time. Repeat functions: vital capacity 1.4 L. (36 per cent), timed vital capacity 100 per cent in 3 seconds, maximal breathing capacity 33 L. per min. (24 per cent), and walking ventilation 24.6 L. per min.

A. T.

FIG. 6(Left). A 26-year-old woman with far advanced, active tuberculosis of four years' duration. With additional treatment the right apical cavity closed but those on the left did not. A left three-stage seven-rib thoracoplasty was performed. Preoperative pulmonary function studies: Vital capacity 1.58 L. (49 per cent), timed vital capacity 96 per cent in 3 seconds, maximal breathing capacity 51.5 L. per min. (46 per cent).

FIG. 7 (Center). Ten months following thoracoplasty, sputum was still positive for acid-fast bacilli. Vital capacity 1.3 L. (46 per cent), timed vital capacity 90 per cent in 3 seconds, and maximal breathing capacity 37.5 L. per min. (35 per cent). A left pneumonectomy was performed.

FIG. 8 (Right). Roentgenogram four months following resection. Sputum negative for tubercle bacilli. Vital capacity 1.3 L. (46 per cent), timed vital capacity 97 per cent in 3 seconds, and maximal breathing capacity 34.6 L. per min. (33 per cent). Patient is still receiving chemotherapy and is doing light housework.

TABLE 3

EARLY RESULTS OF SURGERY

Maximal Breathing Capacity 50 Per Cent or Below

Maximal Breathing Capacity	Survived		Died	
	Collapse	Resection	Collapse	Resection
per cent				
20–24	1 thoracoplasty 1 plombage			
25–29	5 thoracoplasties 1 plombage	2 lobectomies	2 thoracoplasties	1 lobectomy
30–34	3 thoracoplasties 1 plombage	2 lobectomies 1 segmentectomy		3 pneumonectomies
35–39	6 thoracoplasties 2 plombages	1 pneumonectomy 3 lobectomies 1 segmentectomy	1 plombage	1 lobectomy
40–44	1 thoracoplasty 3 plombages	1 pneumonectomy 13 lobectomies 8 segmentectomies		1 segmentectomy
45–50	6 thoracoplasties 2 plombages	7 pneumonectomies 24 lobectomies 5 segmentectomies	1 plombage	1 pneumonectomy 1 lobectomy 1 segmentectomy

has been denied surgery because it is believed that the risk involved is too great. Three patients, still with active disease, are at home and are classified as having received the maximal benefits of therapy.

DISCUSSION

Tuberculosis is a destructive disease which, even with healing, often results in extensive damage to the lung. Pulmonary function studies have been useful in evaluating the extent of tissue damage as well as in reflecting the decrease of ventilation from such conditions as pleural fibrosis, weakness or paralysis of the muscles of respiration, and general physical debility (1). Of the various tests available, probably the maximal breathing capacity indicates a reduction of these factors better than any other single test. In this study it has been impracticable to give data relevant to the other screening function tests, and in actuality these tests failed to correlate with surgical risk as well as maximal breathing capacity.

Various function studies singly or in combination have been used in an effort to estimate which patient should be treated and which patient should be refused surgery. Warring (2) recommended the determination of the walking ventilation and the maximal breathing capacity in patients being considered for collapse procedures, and stated that thoracoplasty should not reduce

TABLE 4

SURGICAL PROCEDURES, 1951–1959

	Patients with Maximal Breathing Capacity Below 50 Per Cent		Patients with Maximal Breathing Capacity Above 50 Per Cent		Total Procedures	
	Number	Mortality	Number	Mortality	Number	Mortality
		per cent		*per cent*		*per cent*
Pneumonectomy	13	30.8	60	8.3	73	12.3
Lobectomy	47	8.5	253	1.6	300	2.7
Segmental resection	17	11.8	321	1.9	338	2.4
Thoracoplasty	24	12.5	22	4.5	46	8.7
Extraperiosteal plombage	12	16.7	6	0.0	18	11.1
Miscellaneous	8	12.5	33	3.0	41	4.9

TABLE 5

PNEUMONECTOMY COMPLICATIONS AND DEATHS

	Maximal Breathing Capacity Below 50 Per Cent		Maximal Breathing Capacity Above 50 Per Cent	
	Number	Died	Number	Died
Patients	13	4	60	5
Complication:				
Bronchopleural fistula and empyema	2	1	10	3
Spread of tuberculosis	2	1	4	1
Hemorrhage	1	1	1	0
Pneumonia (viral)	0	0	1	1
Pulmonary edema	1	1	0	0
	6		16	
Per cent of complications	46		27	
Mortality following complication	64%		31%	

the maximal breathing capacity below a value of twice the walking ventilation. Thomas and Steele (3) suggested that surgical collapse therapy should not be advised in those patients in whom the ratio of walking ventilation to the maximal breathing capacity exceeds 0.35. Morton and Khan (4) recently discussed resection in relation to preoperative ventilatory function studies. They suggest that any patient with a maximal breathing capacity above 80 per cent does not require any further screening tests and that this test alone is adequate in 85 per cent of their patients. For those patients with maximal breathing capacities below 80 per cent, they recommend that additional methods of evaluation be done. While these writers believe that the maximal breathing capacity below which surgery should not be performed is approximately 40 per cent, the patient with the lowest value in whom surgery was done in their study had a determination of 45 per cent of the predicted value. This patient, treated by a pneumonectomy, died.

It would be convenient to have some objective test that at a certain level of depression would contraindicate surgery. Attempts have been made to use the ventilatory function tests in this manner. Early experience at the Ohio Tuberculosis Hospital with pneumonectomy in patients with maximal breathing capacities below 50 per cent suggested that these patients should be refused surgery. However, the known tolerance of these patients to collapse procedures and the increased ability of post-thoracoplasty patients to withstand resection stimulated return to the use of pre-pneumonectomy thoracoplasty. This practice had two results: First, of the 20 patients in whom pneumonectomy was considered to be indicated but in whom thoracoplasty was performed first, only 5 required subsequent resection; in 15 the disease was controlled by the collapse procedure alone. Second, there was a tendency to believe that any patient with a maximal breathing capacity of 50 per cent or below was an extremely hazardous surgical risk. While this latter statement may be true, surgical therapy can be accomplished in many of these patients with only a moderate increase in risk. Surgical treatment in many of these patients represented salvage surgery, and the mortality rate and complication rate are appreciably higher than in patients with maximal breathing capacities above 50 per cent; yet, collapse therapy has been done successfully in patients with maximal breathing capacities as low as 22 per cent, and lobectomy has been done in patients with maximal breathing capacities as low as 28 per cent. This indicates only that, in patients in whom other factors are favorable, a maximal breathing capacity at these levels should not be considered an absolute contraindication to surgery. When an operation is performed on a lung that has little or no pulmonary function, the patient is not deprived of functioning pulmonary

TABLE 6

LOBECTOMY COMPLICATIONS AND DEATHS

	Maximal Breathing Capacity Below 50 Per Cent		Maximal Breathing Capacity Above 50 Per Cent	
	Number	Died	Number	Died
Patients	47	4	253	4
Complication:				
Bronchopleural fistula and empyema	9	1	21	0
Spread of tuberculosis	1	1	4	0
Hemorrhage	0	0	8	2
Pneumonia	1	1	2	1
Transfusion reaction	1	1	0	0
Cerebral vascular accident	0	0	1	1
	12		36	
Per cent of complications	24		14	
Mortality following complication	33%		11%	

TABLE 7

CURRENT STATUS—111 PATIENTS

Maximal Breathing Capacity 50 Per Cent or Below

Procedure	Number	Inactive		Active Tuberculosis	Death	Unknown
		Working	Not Working			
Pneumonectomy	13	4	4	0	5	0
Lobectomy	44	13	22	3	4	2
Segmental resection	15	4	6	1	3	1
Thoracoplasty	22	7	5	3	6	1
Extraperiosteal plombage	11	1	2	3	4	1
Miscellaneous	6	2	1	1	1	1
	111	31	40	11	23	6
Per cent of total		64		10	20.7	

tissue and, in some cases of pneumonectomy, when the destroyed lung has been acting as a huge atrial-venous shunt, patients are improved after pneumonectomy.

It must be stated that, while the maximal breathing capacity of 50 per cent or below has been used as the method of selection for these 111 patients at the Ohio Tuberculosis Hospital, this may not be a valid level of selection in other patients, since techniques of performing pulmonary function tests vary in different institutions. However, when this test is performed in the laboratory at the Ohio Tuberculosis Hospital a maximal breathing capacity below 50 per cent appears to signify increased surgical risk. It must further be stated that the conclusions derived from this present study apply only to pulmonary tuberculosis and that these figures cannot be applied to other pulmonary diseases, such as bronchogenic carcinoma, in which lobectomy and pneumonectomy have been performed successfully at lower levels of preoperative pulmonary impairment than in patients with pulmonary tuberculosis.

SUMMARY

Between 1951 and 1959, 111 patients with pulmonary tuberculosis who had preoperative maximal breathing capacities below 50 per cent were treated surgically at the Ohio Tuberculosis Hospital. Thoracoplasties were performed successfully in patients with maximal breathing capacities of 22 per cent, and resection in those with maximal breathing capacities of 28 per cent. The complication and mortality rates in these "poor-risk" patients are reviewed and compared with those in a group of patients with maximal breathing capacities above 50 per cent.

The experience delineated in this report should not be interpreted as an attempt to extend the use of surgery, nor is the fact that surgical therapy has been accomplished successfully in a majority of these patients with markedly reduced pulmonary function intended as an attempt to stimulate a comparable surgical attack on other "poor-risk" patients. Rather, this study has been done to re-emphasize that pulmonary function studies are a laboratory method designed to give the clinician an estimate of pulmonary reserve. No patient should arbitrarily be refused consideration for surgical therapy because pulmonary function is depressed. Only when these studies are correlated with the clinical status of the patient do they achieve validity.

SUMARIO

La Cirugía por Tuberculosis Pulmonar en el Enfermo de Función Ventiladora Atenuada

Entre 1951 y 1959 se trató quirúrgicamente en el Hospital de Tuberculosis del Estado de Ohio a 111 enfermos de tuberculosis pulmonar que tenían preoperatoriamente capacidades respiratorias máximas de menos de 50 por ciento. Se ejecutaron con éxito toracoplastias en los enfermos que tenían una capacidad respiratoria máxima de 22 por ciento, y la resección en los que tenían una capacidad respiratoria máxima de 28 por ciento. Se repasan las tasas de complicaciones y mortalidad en esos enfermos, que son "malos riesgos," comparándolas con las observadas en un grupo de enfermos que tenían una capacidad respiratoria máxima de más de 50 por ciento.

No debe interpretarse la experiencia delineada

en esta comunicación como tendiendo a extender el uso de la cirugía, ni tampoco el hecho de haberse ejecutado con éxito la terapéutica quirúrgica en la mayoría de estos enfermos cuando tenían una acentuada reducción de la función pulmonar como esfuerzo encaminado a alentar un ataque quirúrgico comparable en otros enfermos que son "malos riesgos." Más bien se ha llevado a cabo este estudio para recalcar de nuevo que los estudios de la función pulmonar son una técnica de laboratorio destinada a suministrar al clínico un cálculo de la reserva pulmonar. A ningún enfermo debe rehusársele arbitrariamente la terapéutica quirúrgica porque tenga la función pulmonar deprimida. Unicamente cuando se correlacionan estos estudios con el estado clínico del enfermo es que adquieren validez.

Resume

Chirurgie pour tuberculose pulmonaire chez le malade ayant une fonction ventilatoire diminuée

Entre 1951 et 1959, 111 malades avec une tuberculose pulmonaire, qui avaient une capacité maxima de respiration de moins de 50% avant l'opération, furent traités chirurgicalement à l'Hôpital de la Tuberculose de l'Ohio. Des thoracoplasties furent pratiquées avec succès chez des malades ayant une capacité maxima de respiration de 22% et une résection chez ceux avec une capacité maxima de respiration de 28%. Les taux de complications et de mortalité, chez ces malades considérés comme de mauvais cas, sont revus et comparés avec ceux d'un groupe de malades ayant des capacités respiratoires maxima de plus de 50%.

L'expérience relatée dans ce rapport ne devrait pas être considérée comme un essai d'étendre l'emploi de la chirurgie, et, le fait que la thérapeutique chirurgicale a été accomplie avec succès chez la plupart de ces malades porteurs d'une fonction pulmonaire diminuée ne tend pas non plus à inciter à une attaque chirurgicale pareille chez d'autres malades étant d'aussi mauvais cas. Cette étude a plutôt été faite pour souligner à nouveau que les études de la fonction pulmonaire sont une méthode de laboratoire conçue pour donner au clinicien une estimation de la réserve pulmonaire. Aucun malade ne devrait être écarté systématiquement de la thérapeutique chirurgicale parce que sa fonction pulmonaire est diminuée. Ces études ne sont valables que lorsqu'elles sont rattachées à l'état clinique du malade.

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A STUDY OF POST-HOSPITALIZATION TREATMENT OF TUBERCULOSIS IN RURAL WESTERN NEW YORK[1]

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Introduction

A number of reports of extra-hospital treatment of tuberculosis have appeared in recent years. Robins and his co-workers (1–3), for example, have reported the results of treatment of unhospitalized patients in New York City; and Silverman (4) has given a report on reduction of mortality in Baltimore during a period when unhospitalized patients were treated there. Stradling (5) and McKay (6) have reported on the results of treatment of patients whose therapy was begun in a clinic; and Forgacs (7) has discussed the place of short-term hospitals in the treatment of pulmonary tuberculosis. Lichtenstein (8) and Bundy and associates (9) have reported the results of treatment of patients in Chicago and Detroit, respectively, who had periods of chemotherapy in the hospital, then continued on an ambulatory basis.

These reports have in common a discussion of home treatment of tuberculosis in situations in which patients are concentrated in a relatively small area, where the problem of distance should not be a large factor in the success of treatment and follow-up procedures. No reports have been found of the home treatment of tuberculous patients scattered over a rural area; this paper describes experiences with the post-hospitalization treatment of such a group.

The observations made here should be regarded as a study, rather than a final demonstration; the results are promising but a longer period of follow-up observation of the patients, particularly those who last entered the study, will be needed before truly valid prognostic conclusions can be reached. The study is being continued for this reason.

Materials and Methods

Setting of Study

The area served by this hospital comprises 14 counties in western New York. All are predominantly rural; their total population in 1950 was approximately 640,000. The maximal distance from the hospital is about 120 miles. About 230 field clinics are held in the area each year and, in the hospital, approximately 140 outpatient clinics are held each year. All are on a consultative basis; patients must be referred by their personal physicians for diagnostic work and whatever follow-up service is deemed necessary. The physicians receive reports describing what has been accomplished after each patient visits the clinic. In 1958, about 11,200 patients had some 13,600 examinations in these clinics.

The field work for the clinics is done by district, state, and local health departments through public health nurses, who periodically visit patients who are under observation. The nurses remind patients when they are due for clinic visits, urge them to attend, and help in any way possible, even to the extent of arranging transportation, to get the patients to clinics on time.

Each field clinic is in charge of a physician, who goes to the clinic from this hospital with an X-ray technician and a portable 14- by 17-inch X-ray unit. In addition to these personnel, public health nurses and volunteers serve as clerks, receptionists, et cetera. Each clinic patient supplies identifying and historical data, which are recorded on a standard clinic form. Whatever physical examination is indicated is done and recorded. A tuberculin test, if not previously positive, is obtained. A chest roentgenogram is routinely taken. Films are taken back to the hospital, developed and read, and reports are sent with the clinic physician's recommendations to the patients' referring physicians.

In the event of need for procedures which cannot be done in the field clinics, patients are brought to the hospital or outpatient clinic, where facilities are available for complete study.

The outpatient chemotherapy program was merely adapted to this basic structure. Inpatient and outpatient treatment for tuberculosis is free in New York State.

Principles of Management of Patients

The basic features of the chemotherapy program are as follows: (*1*) a prior period of hospitalization; (*2*) a definite agreement between physician and patient, noted in the hospital discharge summary or clinic record (as the case may be), stating the time and place for the next meeting with the patient; a clinic schedule is carried to all clinics and consulted in making a choice of date and place; (*3*) a chest roentgenogram each time drugs

[1] From the Mount Morris Tuberculosis Hospital, Mount Morris, New York; and the New York State Department of Health.

are dispensed (approximately every fifty-six days); (*4*) return of drugs to the hospital if the patient fails to appear at the agreed-upon clinic; (*5*) periodic evaluation of each case in staff conferences.

It is pertinent to discuss the rationale of these features of the program. In the first place, comment should be made on the period of prior hospitalization. Such a period of hospitalization is indicated because it keeps the patient away from the public until tubercle bacilli have been eliminated from his secretions and it provides opportunity for the best evaluation of the patient's disease through day-to-day observation. Moreover, establishment of the therapeutic regimen is safer if the patient is under daily observation during the early period of treatment, when most drug reactions occur and can be recognized and controlled quickly. Rehabilitation needs can best be evaluated during a period of daily contact with the patient. In the beginning it was thought that a minimal period of hospitalization should be four months, but for many patients it was found that this time could be reduced. Many, of course, require more than four months' hospitalization for public health and/or physical reasons.

At the beginning of the study each patient was required to come to the hospital outpatient department every twenty-eight days for drugs, questioning, and examination. This was convenient for the staff, and made simple the delivery of drugs to patients. The relatively short interval was chosen because the possible difficulties which might arise as a result of administration of potentially toxic drugs were overestimated. It was feared that moral responsibilities to the patient could not be discharged if they were seen less often. Further rationalization made it seem just as logical to ask the patient to return to the outpatient department every twenty-eight days for drugs as it had been to ask him to return for pneumothorax refills in the pre-chemotherapy days.

It soon became evident, however, that few patients had symptoms; this fact resulted in perfunctory questioning and examination of the patient by the physician. It was only natural that the patient should resent driving many miles for what appeared often to be trivial reasons. Also, welfare agencies, sometimes called upon to transport indigent patients, could not understand spending scarce time and money for that purpose. Drug reactions were few and not serious, and it became clear that an interval between visits of two months (or longer, in some instances) was safe and more convenient for everyone than the original twenty-eight day interval.

TABLE 1
PATIENTS TREATED

Pulmonary tuberculosis	339
Extrapulmonary tuberculosis	57
Primary tuberculosis	13
Total	409

Sometimes a patient telephoned to say that he was unable to get to the clinic, but that he felt well and desired that a supply of drugs be mailed to him. Such calls were likely to come on days when the patient was scheduled for a new supply of drugs but not for a chest roentgenogram; when he was due for a chest film he usually appeared, and it was clear that the roentgenogram was some sort of enticement as far as coming to the clinic was concerned. This was another reason for lengthening the interval between visits and requiring a chest film each time.

The definite agreement between physician and patient about the time and place of each visit was necessary, of course, to make certain that patient and drugs reached the same clinic. It was obvious that the day-to-day handling of such details depended not only on the patient, clinic personnel, and public health nurses, but also on a coordinator within the hospital. The head nurse in the outpatient department was chosen as coordinator. She got drugs to patients on schedule with the help of a simple tickler file. As might be expected, she sometimes received requests for revisions in the agreement previously made between patient and physician, but the mechanical aspects of the program functioned surprisingly well.

Criteria for Admission to Outpatient Chemotherapy Program

The following criteria were fulfilled before patients were permitted their discharge from the hospital to receive outpatient chemotherapy: a suitable drug regimen was established; sputum was negative for tubercle bacilli on microscopy and culture; the roentgenogram was improving or stationary; and the patient was reasonably reliable, with a stable life situation.

Some concessions were made in the application of these criteria from time to time, particularly the interpretation of what constituted a stable life situation. In the main, however, they were followed well.

Characteristics of Group of Patients

The patients who are the subjects of this paper numbered 409. This included all who started outpatient chemotherapy under the auspices of this hospital during the years 1954–1957, inclusive. The type of disease with which the patients suffered is shown in table 1; in instances in which both pulmonary and extrapulmonary tuberculosis were present, the patient is listed under the type of disease which required the longest period of therapy.

The average age of the males was forty-nine and, of the females, thirty-six. The extremes of age were less than one to eighty-eight years for males and less than one to eighty-one years for the females. Two hundred and thirty (56 per cent) were males and 179 (44 per cent) were females. More than 95 per cent were white. During the period covered by the study, the whole group of

TABLE 2

REASONS FOR DISCHARGE—ALL PATIENTS

	MHB*	Personal with Consent	Personal without Consent	Disciplinary AWOL†	Transferred	Dead	Total Pulmonary Disease	Total Primary and Extrapulmonary Disease	Total of All Patients
1954	137	19	31	28	16	21	240	12	252
1955	155	30	23	35	6	23	249	23	272
1956	173	45	24	32	28	20	293	29	322
1957	167	7	14	25	38	26	249	28	277
Totals	632	101	92	120	88	90	1,031	92	1,123

* MHB—maximal hospital benefit
† AWOL—absent without leave

patients discharged from the hospital were comparable, except that males comprised 62 per cent and females 38 per cent of all discharges. It appears that females are somewhat more likely than males to meet the criteria for outpatient chemotherapy.

When the outpatient chemotherapy program was started, it was hoped that the number of premature discharges from the hospital would be reduced. As shown in table 2, during the period of study, premature discharges dropped from 59 (approximately 26 per cent) in 1954, to 39 (about 16 per cent) in 1957. (The term "premature discharge" is used to describe those patients who were discharged for personal reasons without consent, who were discharged for disciplinary reasons, or who were absent without leave. Patients who died were excluded from the total in this calculation.) The change is principally in the group which received discharges for personal reasons, reflecting the willingness of the staff to make concessions to the patient to keep him under treatment. These concessions came more readily as confidence in outpatient chemotherapy increased.

In connection with table 2: If an individual had two or more discharges during the year, only the first was recorded; if the patients in the first two columns, discharged with maximal hospital benefit or for personal reasons with consent, are taken together, 733 patients were theoretically eligible for outpatient treatment during the years of the study; 352 started on outpatient chemotherapy.

An analysis of the patients with pulmonary tuberculosis in this study shows that 22 (8 per cent) had excisional surgery prior to discharge and 19 were readmitted for excisional surgery, so that a total of 15 per cent finally received resections. This compares with a total of 25 per cent of all 1,031 discharged patients with pulmonary tuberculosis who had excisional surgery prior to discharge. Obviously, a number of patients who might otherwise have been eligible for outpatient chemotherapy had a plan of treatment, including excisional surgery, which could most conveniently be completed in the hospital.

RESULTS OF TREATMENT

The present analysis is made as of December 31, 1958. Thus, the maximal length of time a patient may have been observed is five years and the minimal, one year, inasmuch as all patients who started outpatient chemotherapy during the years 1954–1957 are included.

The various chemotherapeutic regimens used will not be discussed in this paper, except to state that isoniazid alone or in combination with *p*-aminosalicylic acid, streptomycin, or cycloserine have all been used. A few patients received streptomycin and PAS alone. A drug regimen was usually determined by the particular regimen under comparative study that was in use locally at a given time.

Of the 409 patients treated, 352 had received their initial therapy in this hospital, 53 had been treated in other hospitals, and 4 had been treated by their personal physicians. Separate analysis shows that final results did not depend on the place where treatment was started.

It seems convenient to deal with the patients who had reinfection pulmonary tuberculosis separately from the patients who had primary tuberculosis or extrapulmonary disease. There were 339 pulmonary disease patients, classified as shown in table 3, which also shows the number of patients started on outpatient treatment during each year of the study.

As shown in table 4, 289 of 339 patients (85 per cent) are known to have achieved inactive status before dying or being lost to observation. Ninety-nine per cent of those who started treatment for minimal disease, 83 per cent of those who began treatment for moderately advanced

disease, and 73 per cent of those who started treatment for far advanced disease reached this classification. Two patients with minimal disease, 14 with moderately advanced, and 19 with far advanced disease are still under treatment.

Eleven patients had sputum positive for tubercle bacilli at the time they were started on outpatient chemotherapy; in 6 the report did not reach the record until after the patient's discharge; and in each of these instances prior reports on sputum had shown it to be noninfectious. The other 5 patients were treated even though they did not meet the criterion of noninfectious sputum for outpatient chemotherapy. This happened because it seemed better, when cogent reasons forced a patient's discharge, to keep him under treatment than to adhere to inflexible principle, with resultant disadvantage to patient and public.

The average duration of hospital treatment for the pulmonary patient who started treatment in this hospital, the average length of outpatient chemotherapy, and the average time required to reach inactive status for those who achieved this were determined. These data are given in table 5.

Clinical considerations and natural caution resulted in treating patients with minimal disease for three months beyond the time when inactive disease had been achieved. (It is believed, on empirical grounds, that a year of chemotherapy should be a minimum.) Patients with moderately advanced disease were treated for an average of five months and those with far advanced disease, for an average of nine months beyond the point when inactive disease was attained. It should be pointed out that these figures are weighted to some extent by difficulties in determining the presence or absence of cavity; in some instances treatment was carried on long after the cavity closed, as determined by tomograms and retrospective reading of chest roentgenograms.

At the time of this writing, 29 (9 per cent) of the patients treated for pulmonary tuberculosis have died. Fifteen had achieved inactive status before death, and 14 had not. The causes of death are set forth in table 6.

Probably tuberculosis was directly or indirectly responsible for the deaths of 8 patients (first 2 categories in table 6). The cardiovascular deaths were: 5 coronary occlusions and 2 cerebral accidents. One of the carcinomas was bronchogenic; the patient achieved inactive status of his tuberculosis despite the fact that his carcinoma was inoperable from the start of his hospital treatment fifteen months before death. The 2 patients who died of unknown causes reportedly died suddenly.

Twenty (7 per cent) of these patients have had relapses of their disease, if one defines relapse as any adverse change by roentgenogram, sputum examination, or symptoms after inactive disease status has been reached. Twelve of the relapses occurred in the first year after inactive status was achieved, and 8 occurred in the second year. Nine of the 20 relapses were manifested by single isolated sputum cultures positive for tubercle bacilli, 4 of the patients had more than one infectious sputum specimen without roentgenographic changes, 4 had infectious sputum and roentgenographic changes, and one had a small hemop-

TABLE 3

YEARS IN WHICH OUTPATIENT TREATMENT STARTED—PULMONARY TUBERCULOSIS

	1954	1955	1956	1957	Totals
Minimal tuberculosis	10	17	40	34	101
Moderately advanced tuberculosis	25	17	56	42	140
Far advanced tuberculosis	18	19	33	28	98
Totals	53	53	129	104	339

TABLE 4

NUMBER OF PATIENTS WITH PULMONARY TUBERCULOSIS WHO REACHED INACTIVE DISEASE STATUS

	1954	1955	1956	1957	Total Inactive	Total Treated	Per Cent Inactive
Minimal tuberculosis	10	17	39	34	100	101	99
Moderately advanced tuberculosis	20	14	49	34	117	140	83
Far advanced tuberculosis	15	14	27	16	72	98	73
Totals	45	45	115	84	289	339	Average 85

TABLE 5

DURATION OF CHEMOTHERAPY, DAYS

Tuberculosis	Minimal	Moderately Advanced	Far Advanced
Hospital	140	234	391
Outpatient	241	322	494
Totals	381	556	885
Time to reach inactive status	286	392	621

TABLE 6

CAUSES OF DEATH—PATIENTS WITH PULMONARY TUBERCULOSIS

Tuberculosis	6
Emphysema and cor pulmonale	2
Cardiovascular disease	7
Carcinoma	7
Pneumonia	1
Suicide	1
Accident	1
Gastrointestinal bleeding	1
Postsurgical hemorrhage	1
Unknown	2
Total	29

tysis which led to the discovery of a late postsurgical bronchopleural fistula. In addition to these relapses, one patient, who had had a thoracoplasty twenty years before, developed a chest wall abscess which arose from the end of a resected rib. It appears reasonably certain that minimal tuberculosis was reactivated in one of the above patients because her lung was invaded with carcinoma, metastatic from a primary site in the rectum.

For the patients with primary tuberculosis a more-or-less standard plan of one year of treatment was followed, although this was extended in 4 instances because continued clearing of pulmonary foci made a longer period of treatment seem desirable. All achieved a satisfactory result of treatment, and none have suffered relapse or died.

The types of disease in the group of patients with extrapulmonary tuberculosis are listed in table 7.

Thirteen of the 18 patients who had tuberculosis of the genitourinary tract have achieved inactive status and the disease has not relapsed; 2 still have active disease and are still under treatment; one was lost to observation without first reaching inactive status; 2 have died, both from renal failure due to tuberculosis of the kidneys.

Twelve of the 13 patients who had tuberculosis of the bone have achieved inactive status and the other still has active disease and continues under treatment. None have died.

All of the 10 patients with tuberculosis of the lymph nodes have achieved inactive status and have finished their courses of therapy.

Eight patients with tuberculous pleural effusions have achieved inactive status and have finished their chemotherapy. No pulmonary lesions are known to have developed in any of these patients; the follow-up study is complete in all but one. One patient with tuberculous empyema developed an obvious bronchopleural fistula, his sputum became persistently positive for tubercle bacilli, and a lesion appeared in his contralateral lung while he was under outpatient drug treatment. He was rehospitalized but, because of his age (sixty-one years) and generally poor physical condition, he has never reached the point where surgical interference seemed feasible.

The 6 patients with miliary and meningeal tuberculosis have all achieved inactive status and have completed their courses of chemotherapy. None have had relapses as far as is known, but 2 have not been seen during 1958. There have been no deaths.

The patient with tuberculosis of the intestines was diagnosed at operation for presumed chronic appendicitis. Rapid relief of symptoms was achieved by drug therapy, and he was arbitrarily given one year of chemotherapy. There has been no recurrence of symptoms.

Drug reactions: Twenty-four patients had untoward reactions to outpatient drugs. Ten were hypersensitivity reactions or vertigo due to streptomycin, 11 were gastrointestinal intolerance to *p*-aminosalicylic acid, 2 were peripheral neuritis

TABLE 7

TYPES OF EXTRAPULMONARY TUBERCULOSIS

Tuberculosis of genitourinary tract	18
Tuberculosis of bone	13
Tuberculosis of lymph nodes	10
Tuberculosis of pleura	9
Miliary and meningeal tuberculosis	6
Tuberculosis of intestinal tract	1
Total	57

caused by isoniazid, and one was a severe anxiety reaction caused by isoniazid. This last patient's anxiety reaction was so severe as to require hospitalization, but it cleared spontaneously in a few days after isoniazid therapy was discontinued.

Follow-up study and patient cooperation: As mentioned above, the longest period of follow-up study was five years and the shortest, one year. A patient was regarded as having been lost to follow-up observation if he was not observed at least once in each year after he started outpatient chemotherapy or if he failed to complete the prescribed course of treatment despite being observed once yearly or more often. In some instances the follow-up data are really reports from another agency to whose area the patient moved after departing from this hospital district. This seems justified because it is the patient's cooperation in treatment and follow-up observation that is important, rather than the place where it is accomplished.

The losses by the year in which outpatient treatment was started (columns) and the year in which the patient was last followed (rows) are shown in table 8. Figures in parentheses are the number of patients started on outpatient treatment each year. The relatively greater number of patients who started outpatient treatment in 1956 and who were last followed in 1957 is explained partially by the fact that most of the outpatient treatment legacies from other hospitals came to us in 1956 as a result of the closure of another state hospital.

Thirty patients, 7 per cent of the total, have been lost to follow-up observation. If the experience with the patients who began outpatient chemotherapy in 1954 is continued with those started in other years, however, the five-year loss will reach 15 per cent. Of the 30 patients lost, 12 are known to have left the hospital district and 18 are thought to be in the district still.

TABLE 8

LOSSES BY THE YEAR IN WHICH PATIENT WAS FOLLOWED

	1954	1955	1956	1957	Totals
1954	0	4	0	5	9 (60)*
1955		2	1	3	6 (63)
1956			1	11	12 (150)
1957				3	3 (136)
Totals	0	6	2	22	30 (409)

* Figures in parenthesis are number of patients who started on outpatient treatment each year.

TABLE 9

BACTERIOLOGIC TESTS—OUTPATIENTS

	1954	1955	1956	1957	1958
1954	48	43	35	29	19
1955		54	44	29	27
1956			110	89	49
1957				92	59
Totals	48 (53)*	97 (103)	189 (221)	239 (316)	154 (280)

* Total number of patients available for bacteriologic examination in any year.

Another measure of the quality of follow-up study may be shown by the frequency with which patients with pulmonary tuberculosis have had examinations of sputum or gastric lavage specimens. It is the custom at this hospital to request a sputum specimen monthly while the patient has active disease; after disease has become inactive, the patient is requested to submit sputum any time productive cough appears or there is a change in the chest roentgenogram. For purposes of assessing patient cooperation and staff performance on this point, table 9 has been constructed. A numerical value of one has been assigned to each patient in each year of follow-up observation if he submitted sputum on an average of once every two months while he had active disease and once yearly after inactive disease status was reached.

Figures in parentheses represent the total number of patients available, i.e., not lost or dead, in any given year to have bacteriologic examinations. Thus, in 1954, 48 of 53 had sputum or gastric examinations according to the schedule outlined above; 97 of 103 had bacteriologic examinations in 1955, and so on. It is realized that this is a very rough measuring device, but it does show that the number of patients who had sputum examinations diminished rather sharply as time went on. Perhaps this is one reason why more relapses were not found, particularly after the second year of inactive disease status. As the records are examined, however, it appears that most of these who needed sputum examinations had such examinations, but this may merely be treacherous self-deception.

The number of patients who interrupted and the number who lapsed treatment were determined. Interrupted treatment was defined as any cessation of chemotherapy, not caused by toxicity or hypersensitiveness, for more than one week and less than four weeks. Any cessation longer than four weeks not caused by toxicity or hypersensitiveness was termed a lapse of treatment. Fortunately, most of the deviations occurred late in the course of treatment. The reasons given by the patients usually seemed trivial. At any rate, 54 (11 per cent) of the patients deviated from the prescribed treatment by their own statement. It must be suspected that more did so, but treatment results were apparently not endangered. Tests for the detection of drug in the urine were not done.

Discussion

A valid way of determining whether final results in the treatment of a large group of patients are affected for better or worse by the availability of an outpatient treatment program would be helpful, but it seems likely that it will be necessary to rely on the judgment of those versed in the treatment of tuberculosis to make this assessment. It is the writer's opinion that, if it were possible to educate all patients to remain in the hospital until they either reached inactive disease status or died, results of treatment would be more nearly optimal than at present. Many patients, however, are unable to bring themselves to such an all-or-none point of view, and it seems far better to give treatment on an outpatient basis than to lose the patient from observation completely because of an inflexible administrative attitude. Whether the criteria and methods for post-hospitalization chemotherapy described in this paper will prove to be as good as those used by other hospitals remains to be seen.

The many reasons why a period of hospitalization should precede the period of outpatient chemotherapy serve to point out the value of the hospital in the scheme of treatment, even though an effort is made to shorten the period of hospitalization as much as possible. Somewhat paradoxically, an outpatient chemotherapy program is one of the devices by which hospitals may be eliminated by reducing the need for beds for tuberculous patients. The fact that there are empty beds in the hospital makes the daily in-hospital cost per patient relatively high, but the total cost of treatment is significantly lower than it would be if all treatment were carried out in the hospital.

It is clear to those who work in this program that good staff teamwork and good rapport with the patients are essential to good results. It seems likely that the weakest feature of the plan presented here is an insufficient number of bacteriologic examinations and, even in this regard, the performance seems adequate, if not ideal. Early outpatient treatment requires a speeding of the rehabilitation process; chemotherapy, in general, reduces the need for vocational rehabilitation by official agencies.

Shortening the period of hospitalization has improved the individual patient's morale and it has improved morale in the hospital generally. There are fewer complaints about food, confinement, and boredom than there once were. Fewer patients are listed as leaving the hospital for personal reasons, but the percentage who receive disciplinary discharges and who are absent without leave remains approximately constant.

Summary

A workable plan for treating tuberculous patients scattered over a wide area of rural western New York has been described and the results of treatment in 409 of these patients, who began post-hospitalization chemotherapy during the years 1954 through 1957, have been presented. Results of the study appear now to indicate that the advantages greatly outweigh the disadvantages, but final prognostic conclusions must await the test of time.

Sumario

Estudio del Tratamiento Post-Hospitalario de la Tuberculosis en Una Zona Rural del Oeste del Estado de Nueve York

Descríbese un plan factible para tratar a los tuberculosos esparcidos a través de una amplia zona rural del occidente del Estado de Nueva York, presentándose los resultados del tratamiento en 409 de estos enfermos que comenzaron la quimioterapia después de la hospitalización durante los años de 1954 a 1957 inclusive. Los resultados del estudio ya parecen indicar que las ventajas contrapesan considerablemente las desventajas, pero que las conclusiones pronósticas definitivas tienen que esperar la prueba del tiempo.

Resume

Une étude du traitement de la tuberculose après l'hospitalisation dans la région rurale à l'ouest de New-york

L'auteur décrit un plan pratique de traitement de malades éparpillés dans une large région rurale à l'ouest de New-york et il présente les résultats de traitement chez 409 malades, qui commencèrent une chimiothérapie après l'hospitalisation, pendant les années allant de 1954 à 1957. Les résultats de cette étude semble indiquer que les avantages l'emportent de beaucoup sur les désavantages, mais, pour porter des conclusions définitives sur le pronostic, il faut attendre l'épreuve du temps.

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VACCINATION AGAINST TUBERCULOSIS WITH NONLIVING VACCINES[1, 2]

III. Vaccination of Guinea Pigs with Fractions of Phenol-killed Tubercle Bacilli

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INTRODUCTION

The ability of intact, killed tubercle bacilli to elicit a state of heightened resistance against subsequent tuberculous infection has been demonstrated repeatedly (1). It has also been reported by a number of investigators that certain isolated moieties of *M. tuberculosis* possess immunogenic capacities, and the accumulated evidence suggests that some fractions may be as effective prophylactically, when they are administered under appropriate experimental conditions, as vaccines composed of living, attenuated, or otherwise nonvirulent organisms (1). The biologic and chemical properties of immunogenic components of tubercle bacilli have remained largely obscure, however, and their reported immunologic activities have not always proved to be readily reproducible in other laboratories. It appears, for several reasons, that further study of the immunogenic behavior of tubercle bacilli should now be directed toward a more definitive isolation and better characterization of active fractions and a standardization of their methods of preparation and testing.

There is the consideration of human protection against tuberculosis. It has been pointed out in a previous discussion (1) that there are persuasive arguments for the continued inclusion of prophylactic vaccination in the arsenal of defenses against the disease, provided that a vaccine free of serious defects could be made available. The most serious faults of vaccines consisting of living tubercle bacilli are their sensitization of recipients to tuberculoprotein, thus causing the loss of the most valuable diagnostic test for tuberculosis, and the possibility, whose exact degree of remoteness has long been a source of heated disagreement, of inducing local or systemic manifestations of the disease which they are intended to prevent. The more cogent objections to the existing living vaccines, BCG and the vole bacillus, are based on these considerations.

Vaccines made of intact killed tubercle bacilli, such as the Anatuberculina Integrale Petragnani (A.I.P.) and the Vaccino Diffondente Antitubercolare Salvioli (V.D.S.) preparations employed for human vaccination in Italy (2, 3), share the same drawback of tuberculin sensitization, and are not entirely free, either, of the possibility of inducing pathologic reactions, directly or indirectly: even killed tubercle bacilli produce injurious effects on the tissues of higher animals. Although the quantities sufficient to cause systemic pathologic changes usually appear to be considerably in excess of optimal immunogenic doses, it is not unlikely that a degree of mild and occult toxigenicity may partially obscure the protective powers of a preparation (4). Even when this is not the case, the persistent local lesions which occur not infrequently at the site of vaccination with some killed preparations are often considered by both patient and clinician as a contraindication to a vaccine. A more subtle, but perhaps more important, element of concern arises from the demonstration that the administration of tubercle bacilli may be followed under certain circumstances by a period of markedly reduced resistance to infection by *other* microorganisms (5).

Vaccines derived from isolated fractions would

[1] From the laboratories of the late A. Q. Wells at the Sir William Dunn School of Pathology, University of Oxford, Oxford, England; and the Department of Bacteriology, University of California, Berkeley.

[2] This study was supported in part by the Medical Research Council of Great Britain and in part by funds from Grant E-2309, National Institutes of Health, U. S. Public Health Service. Some of the experiments were carried out during the writer's tenure of a Merck Senior Postdoctoral Fellowship.

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[4] A large part of these studies was initiated and carried out jointly with Dr. Wells before his untimely and much regretted death in October, 1956. The manuscript was written subsequently, however, and any criticisms directed at the organization, presentation, or interpretation of the experiments must be borne entirely by myself.—D.W.W.

seem to hold the promise of freedom from these objections. There is a strong inclination by some investigators to view hypersensitivity to tuberculoprotein and enhanced resistance to reinfection as independent and unrelated manifestations of infection with tubercle bacilli (6), and a more widely expressed hesitancy to look upon tuberculin *skin* hypersensitivity as a condition or criterion *sine qua non* of immunity (6, 7). Indeed, there have been a number of reports that a degree of protective immunity can be induced in experimental animals by substances extracted from tubercle bacilli which do not simultaneously evoke skin allergy to Old Tuberculin (OT) or purified protein derivative (PPD) (1). Evidence has also been brought forward that the immunogenicity and toxigenicity of tubercle bacilli are independent properties (4). Crude moieties possessing primary toxic but no immunogenic activity (8, 9), on the one hand, or capable of giving some immunity with little evidence of toxicity, on the other (10), have been isolated. It is not inconceivable, therefore, that a more purified fraction of tubercle bacilli can be developed which would retain the intact organism's immunizing potential, but be free of those other properties which make it undesirable as a vaccine.

The development of such a material and the standardization of its activity are not only likely to be of considerable practical interest, but may also contribute to the study of the mechanisms of immunity to tuberculosis. The mode of action of acquired resistance to tuberculosis has remained obscure despite intensive study, and some of the basic questions, such as the relationship of tuberculin hypersensitivity and the presence of certain humoral antibodies to immunity, remain almost as open today as they did in the years immediately following the discovery of the tubercle bacillus by Koch (11). The difficulty of the problem lies, obviously, in the complexity of the host-parasite relationship in tuberculosis. Infection with intact tubercle bacilli, living or dead, evokes simultaneously a large number of host responses. It is exceedingly difficult to separate those which have a true protective function from those which are only incidental manifestations of their presence. The most promising approach lies in the study of the immunologic activities of separate components of the tubercle bacillus, each one capable of evoking only a limited number of host reactions.

Current developments re-emphasize the validity of an intensive study of both the specific and the nonspecific immunologic activities of isolated components of the tubercle bacillus. It has long been recognized clinically that the outcome of one infectious disease may be influenced profoundly by another concomitant, previously established, or superimposed infection. This impression had already won some experimental confirmation in the past (12-14), and has come under intensive investigation in recent years (*see* 5, 15–39). The result has been an increasing recognition that certain bacterial substances can profoundly alter resistance to infections caused by, or to toxic substances produced by, heterologous organisms. The lipopolysaccharide component of the endotoxins of gram-negative bacteria and certain fractions of tubercle bacilli have been especially incriminated in such nonspecific activity. The practical implications of these discoveries for clinical and preventive medicine are evident. They also suggest that the increased resistance against *homologous* reinfection which accrues to survivors of a first infection, or which results from a specific immunization procedure, may not always be based on specific immunologic reactions, i.e., those involving antibody or tissue hypersensitivity, but may be due, at least in part, to the same nonspecific effects which underlie the development of heterologously oriented changes in immunity. This consideration immediately recommends a broad, comparative approach to the elucidation of certain aspects of states of specifically acquired immunity which have defied definition in terms of the classic concepts centered on antibody. Thus, it may be hoped that an understanding of the modes of action of nonspecific immunity will prove to be of value to the analysis of specifically acquired resistance.

Because of the obscurity of the mechanisms of resistance to tuberculosis, and the experimental indications that the tubercle bacillus can affect appreciably the responses to other pathogens, attempts to unravel the elements of specificity and nonspecificity of immunity which may be induced by this organism are of special interest. The most promising experimental approach appears to lie in the determination of whether the bacterial fractions responsible for its effects on resistance toward homologous infection are identical with, or distinct from, those which exert effects against other pathogens. This approach

necessitates the prior establishment of the degree of immunogenic activity of bacterial fractions vis-a-vis *homologous* disease, in this case tuberculosis. Although, as has been pointed out, various moieties of the tubercle bacillus have been shown to possess immunogenic activity, there is little information available—and still less agreement—regarding the conditions and parameters of this activity. In providing a baseline of comparison for the study of the nonspecific activities of the tubercle bacillus, the better definition of the immunogenic activities of its fractions against tuberculosis has implications that go beyond the immediate problem of tuberculosis immunization.

A variety of chemical and physical methods have been employed in the past to obtain immunogenically active fractions of tubercle bacilli (1). A commonly used procedure has been to subject the intact bacillus to differential extraction with aqueous and organic solvents. Active material has been recovered by treatment with different solvents, but it is not clear whether the bacillus contains different immunogenic moieties possessing individual solubility characteristics or whether a similar moiety, soluble in a variety of solvents, has been obtained by the several extraction techniques reported. A logical course of investigation to arrive at an immunologic characterization of the bacillus would be to undertake its systematic chemical breakdown, for example, along the lines followed by Anderson (40); and to test its various components under similar experimental conditions, with appropriate experimental variables (4) and in statistically significant numbers of animals. Mundane considerations of time, space, and funds unfortunately often conspire to make logic a more or less Utopian ideal in tuberculosis studies, however, and a convincing systematic study of the immunogenicity of tuberculofractions has yet to be undertaken.

The present communication is limited to experiments with two fractions of the tubercle bacillus. The purpose of this very limited study was to obtain a somewhat better definition of the behavior and conditions of activity of *some* immunogenic fractions in a species of animal in which tuberculin hypersensitivity can be conveniently measured, with the hope that such information would facilitate the more methodical investigation which should and, it is hoped, will be undertaken sometime in the future. The two fractions studied were a methanol extract of phenol-killed tubercle bacilli and the residue of methanolic extraction. The extract was chosen because a similar material was among the first fractions of tubercle bacilli to be subjected to intensive study and to be shown to have some resistance-enhancing properties (41–43); a recent reinvestigation of the immunogenic activity of methanol extracts has shown them to be highly effective in mice (10, 44, 45). It was also found in earlier experiments (10, 46) that even exhaustive methanol extraction did not remove the immunogenic capacity of intact, phenolized tubercle bacilli and that the addition of small amounts of residue to the methanol-soluble fraction enhanced the extract's immunogenic efficacy. This retention of resistance-affecting activity by a bacterial residue after prolonged extraction with a solvent which removes a similarly active moiety bears a resemblance to the observation that the cell walls of gram-negative bacteria still retain endotoxin-like immunologic activity after chemical removal of their immunologically potent endotoxin (lipopolysaccharide) fractions (47). There are a number of other suggestive similarities between the nonspecific immunologic activities of certain particulate moieties of mycobacteria and enterobacteria (48), and the residues of methanol extractions of tubercle bacilli were therefore investigated further to establish their homologous immunogenicity as well as to provide a basis of comparison for future studies of their nonspecific action (39).

Experimental Methods

The methods, procedures, animals, and materials employed in this study were the same as already described in the preceding communication (4).

Preparation of methanol extract and extraction residue: The extracts were prepared from phenol-killed tubercle bacilli of either the BCG-Phipps strain or of a mixture of the virulent human strains "PN," "DT," and "C," employed at the Veterinary Laboratory of the Ministry of Agriculture, Fisheries, and Food at Weybridge, England, for the production of tuberculin. The origin of these strains, the methods of cultivation, and the procedure of killing with phenol have already been detailed (4).

Quantities of 250 to 800 ml. of absolute methyl alcohol, reagent grade, were added to amounts of the phenolized and dried bacillary mass ranging from 3 to 22 gm. (dry weight). The mixture was placed in a round-bottom flask, to which a water-cooled reflux condenser was attached. The flask was then set into a water bath whose temperature

was maintained thermostatically at 55° C., with variations not exceeding 0.4° C. The water bath rested on a magnetic stirrer, and the bacillary suspension in the flask was kept in a state of constant agitation by means of a Teflon-coated magnetic bar. The bacilli were extracted on eight to twelve consecutive days for periods of six to thirteen hours each. At the end of each period, the alcohol was decanted through two layers of fine-grade filter paper; the glass funnel was placed in a second, water-jacketed metal funnel, heated to approximately 75° C. to prevent the precipitation of extract in the process of filtration. Quantities of fresh methyl alcohol were then added to the bacillary residue, and the mixture was placed in the cold until further extraction was performed on the following day.

The methanol solution after extraction was slightly opalescent while warm. The degree of turbidity increased rapidly upon cooling, and a white amorphous precipitate appeared after several hours at room temperature. More flocculent material precipitated when the solution was set into a refrigerator at 4° C. Microscopic examination of the filtrate revealed a slightly acid-fast mass in which discrete bacterial bodies or fragments could not usually be distinguished. Occasional samples of the extract did, however, appear to contain small quantities of bacterial debris, and it was thought possible that variations in the degree of the tuberculin-sensitizing and immunizing powers of different lots of extract might be due to differences in the extent of contamination by bacillary fragments. In order to remove all traces of such material, the methanolic extract solutions obtained in some of the experiments were filtered under positive pressure (4 pounds per square inch) through special filters having a porosity of 0.2 to 0.3 microns. The filters employed for this purpose were the "cella-filter" type.[5]

The temperature of the solutions during filtration was thermostatically regulated and maintained at approximately 50° C. The filtrate obtained after one or two passages was identical in gross appearance to the unfiltered parent solution. Filtration did not measurably decrease the solutions' content of soluble extract, but appeared to remove most particulate microbial debris, as indicated by the recovery of small amounts of such material from the surface of the filters, and an inability to find it, by microscopic examination, in the filtrate.

To recover the methanol-soluble fraction, the pooled alcoholic solutions were evaporated to dryness in a round-bottom flask under vacuum at a temperature not exceeding 19° C. The residue was an amorphous, amber-colored, "waxy" substance, which became more deeply colored and brittle upon standing in air for more than half an hour. In order to avoid the possibility of a change of biologic activity with this change in appearance, the extract was immediately suspended in sterile distilled water after the methanol had been evaporated; the aqueous suspension was dispersed by means of a Teflon tissue-grinding apparatus (49) and stored in the cold until used.

The residue of methanol extraction was recovered by air-drying the material at room temperature. It was then ground to a fine powder in a porcelain mortar and stored in the cold. Microscopic examination revealed the residue to consist of a mixture of intact bacterial bodies and bacterial fragments and debris; it retained the acid-fast staining property, but to a weaker degree than unextracted phenol-killed tubercle bacilli.

Evaluation of results: As in the previously published trials with intact phenol-killed tubercle bacilli (4), the animals in all experiments were permitted to live to term after the experimental infection. The day of death of each animal was recorded and the mean survival time of each group was calculated. An immediate indication of the degree of survival prolongation afforded by the several preparations is thus provided by a comparison of the mean survival times of vaccinated animals with those of the water-injected controls which were included in every experiment. However, in view of the unequal degree of variability in the survival times of animals in the several vaccination groups of each experiment, and a tendency toward skewness and, hence, non-normality of the data, *t*-tests were not here considered to be a suitable analysis of the statistical significance of survival time differences. Instead, a distribution-free test, the Mann-Whitney test, was applied. As previously (4), the significance of the comparisons of experimental with control groups are expressed as P values. The writer wishes to express his appreciation to Mr. P. S. Puri and Dr. Wm. Taylor of the Department of Biostatistics of the School of Public Health of the University of California in Berkeley for performing the statistical evaluations.

Results

Immunogenicity and Allergenicity of Methanol Extracts

Experiment 1: Groups of 8 to 10 guinea pigs each were vaccinated with one of the following substances: 3.0 or 10.0 mg. (dry weight) of a nonfiltered, heated (70° C. for fifteen minutes) methanol extract preparation derived from phenol-killed BCG-Phipps organisms, intraperitoneally in 1.0 ml. of distilled water; living BCG of a lyophilized preparation made at the Pasteur Institute, Paris, and found to contain 6×10^6 living bacilli per ml., 1.0 ml. subcutaneously; and distilled water, 1.0 ml., intraperitoneally. Thirty-three days later, the animals were skin-tested by an intradermal injection of 0.2 ml. of a 1:100 dilution of a concentrated solution of Weybridge mammalian Old

[5] Manufactured by the Membranfiltergesellschaft of Göttingen, Germany, and made available through the kindness of the Hudes Merchandising Corporation, London.

Tuberculin. (This inoculum contained 200 tuberculin units (TU) (50).) The skin reactions of the animals were observed for a period of 48 hours. The animals were then infected by an intraperitoneal injection of 1.0 ml. of a 1:100 dilution of a seven-day-old Tween®-albumin medium culture of the virulent Branch strain of bovine tubercle bacilli. The size of the preinfection tuberculin reactions and the postinfection survival times of the animals are shown in table 1.

The survival data presented in table 1 show that vaccination with methanol extract prolonged appreciably the survival time of the animals after experimental infection, but that the degree of this protection was not as great as that produced by living BCG. The smaller quantity of vaccine, 3.0 mg., appeared to be more effective than the larger amount, 10.0 mg., a finding similar to that obtained in previous experiments with such extracts in mice (10) and with intact killed bacilli in guinea pigs (4). Most of the animals which received the smaller quantity of extract failed to react to 200 TU of OT when tested immediately prior to challenge infection. That the extract still retained some sensitizing ability, however, was seen from its sensitization of all the animals given the larger amount. It was apparent, nonetheless, that no direct relationship existed between the degree of tuberculin skin hypersensitivity and the degree of protective immunity, as measured by length of survival after infection, which was produced by this preparation.

Experiment 2: Groups of 8 guinea pigs each were vaccinated by either the intraperitoneal or the subcutaneous route with different quantities of nonpasteurized, freshly made methanol extract. Some animals received extract obtained from phenol-killed bacilli of the BCG strain, and others similarly prepared material of human origin. As indicated in table 2, the extracts were given either in distilled water or in incomplete Freund's adjuvant. Two groups of animals received aqueous suspensions of methanol extract mixed with small quantities of the extraction residue or with a purified polysaccharide preparation of the somatic, endotoxin antigen of a Shigella organism. Control groups of animals received distilled water, Freund's adjuvant, extraction residue, or the Shigella polysaccharide. A group of 12 animals was immunized with living BCG. Eight weeks after vaccination, all animals were skin-tested by an intradermal injection of 0.2 ml. of a 1:100 dilution of a concentrated solution of Weybridge mammalian PPD, containing 200 TU. The skin reactions were observed for forty-eight hours. The animals were then infected by an intramuscular injection, in the right thigh, of 0.5 ml. of a 1:100 dilution of a seven-day-old Tween-albumin medium culture of the Branch strain of tubercle bacilli. The size of the preinfection tuberculin reactions and the postinfection survival times are presented in table 2.

It may be seen from table 2 that methanol extract alone, regardless of quantity, strain origin, or route, was immunogenically inactive. When the extract was emulsified in Freund's adjuvant, a slight degree of protective activity was seen. The combination of small quantities of extract and extraction residue was as effective as the living BCG vaccine; here, again, an increase in the quantity of a vaccine was seen to greatly reduce its efficacy, even though the larger amount did not give rise to post-vaccination symptoms of toxicity.

Comment: The difference in the results obtained in *Experiments 1* and *2* with methanol extracts administered without an adjuvant is typical of the observations made in subsequent studies. Occasionally, the extract seemed to be almost as effective a protective agent as living BCG and, at other times, to be completely inert. This variability appeared, at first sight, to be explainable on the basis of certain differences in experimental design. The length of the vaccination-challenge intervals was considered to be of special importance in view of the report by Nègre (43) that a similar methanol extract of tubercle bacilli elicited a state of protective immunity, the duration of which did not exceed a period of approximately two months. Repeated further experiments did not support this impression, however. At times, vaccination with methanol extracts produced an increase in resistance to experimental tuberculosis which was still noticeable when infection took place two or three months after vaccination; but more frequently the extract was entirely ineffective regardless of whether challenge took place several weeks or some months later.

Another possible reason for the low degree of protective activity exhibited by methanol extracts in many experiments might have been the severity or route of the challenge infection. There is always the possibility that a vaccine might offer significant protection against infection with a limited number of pathogenic organisms, but prove to be impotent in protecting against very massive, overwhelming infection. Such a vaccine might still be very useful for man, whose naturally

TABLE 1

TUBERCULIN SKIN HYPERSENSITIVITY AND POSTINFECTION SURVIVAL TIME OF GUINEA PIGS VACCINATED WITH METHANOL EXTRACT OR LIVING BCG

Vaccine	Number of Animals	Tuberculin Skin Hypersensitivity 33 Days after Vaccination: Average Diameter of Induration at Site of Injection of Each Animal after 48 Hours* (mm.)	Days of Survival of Each Animal following Intraperitoneal Challenge Infection 5 Weeks after Vaccination	Mean Survival Time (days)	Per Cent Increase of Mean Survival Time over Distilled Water Controls	Statistical Significance of Comparisons of Survival Times of Vaccinated with Control Groups (Mann-Whitney Test) (P)
Methanol extract, 3.0 mg., intraperitoneally	8	0 0 0 0 0 0 3 7	30 34 34 37 39 40 92 136	55	67	0.003
Methanol extract, 10.0 mg., intraperitoneally	8	10 10 12 13 15 15 18 18	23 29 34 34 34 36 46 50	36	9	0.029
Living BCG†, 1.0 ml., subcutaneously	10	12 14 15 17 17 17 18 18 20 22	39 39 53 53 60 64 65 65 85 110	63	91	<0.01
Distilled water, 1.0 ml., intraperitoneally	8	0 0 0 0 0 0 0 0	19 20 22 25 25 29 32 91	33	—	—

* Tuberculin test with 200 tuberculin units of Old Tuberculin.

† Reconstituted suspension of a freshly obtained lyophilized preparation containing 6×10^6 living organisms per ml.

TABLE 2

TUBERCULIN SKIN HYPERSENSITIVITY AND POSTINFECTION SURVIVAL TIME OF GUINEA PIGS VACCINATED WITH METHANOL EXTRACT, METHANOL EXTRACT IN COMBINATION WITH OTHER SUBSTANCES, OR LIVING BCG

Vaccine	Number of Animals	Tuberculin Skin Hypersensitivity 8 Weeks after Vaccination: Average Diameter of Induration at Site of Injection of Each Animal after 48 Hours* (mm.)	Days of Survival of Each Animal following Intramuscular Challenge Infection 58 Days after Vaccination	Mean Survival Time (days)	Per Cent Increase of Mean Survival Time over Distilled Water Controls	Statistical Significance of Comparisons of Survival Times of Vaccinated with Control Groups (Mann-Whitney Test) (P)
Methanol extract (BCG)						
1.0 mg., IP† in distilled water	8	0 0 0 0 0 0 0 0	52 60 67 77 77 90 102 118	80	8	0.340
3.0 mg., IP in distilled water	8	0 0 0 0 0 0 0 0	52 58 59 65 76 83 90 99	73	−1	0.520
6.0 mg., IP in distilled water	8	0 0 0 0 0 0 0 0	57 62 66 69 70 70 73 77	68	−8	0.139
12.0 mg., IP in distilled water	8	0 0 0 0 0 0 0 0	68 71 72 72 81 84 88 90	78	5	0.399
Methanol extract (BCG)						
1.0 mg., SC‡ in distilled water	8	0 0 0 0 0 0 0 0	59 62 64 79 81 84 91 96	77	4	0.439
3.0 mg., SC in distilled water	8	0 0 0 0 0 0 0 0	56 62 66 71 84 97 101 112	81	9	0.253
6.0 mg., SC in distilled water	8	0 0 0 0 0 2 7 8	55 62 63 73 73 82 90 94	74	0	0.500

Methanol extract (human)																															
3.0 mg., IP in distilled water	8	0	0	0	0	0	0	0	0					63	70	72	75	79	80	84	92					77	4	0.480
6.0 mg., IP in distilled water	8	0	0	0	0	0	0	0	0					69	73	78	83	88	94	98	105					86	16	0.178
12.0 mg., IP in distilled water	8	0	0	0	0	0	0	0	0					54	59	65	73	78	82	106	109					78	5	0.323
Methanol extract (BCG)																															
1.0 mg. + Shigella polysaccharide, 0.05 mg., IP in distilled water	8	0	0	0	0	0	0	0	0					61	68	76	78	79	86	95	96					80	8	0.399
Methanol extract (BCG and human, mixed)																															
1.0 mg., IP in 0.24 ml. Freund's adjuvant	8	1	3	8	11	12	12	15	27					69	71	79	85	85	93	93	141					90	22	0.253
3.0 mg., IP in 0.72 ml. Freund's adjuvant	8	0	1	2	3	5	8	21	22					62	74	80	102	105	117	117	117					97	31	0.047
Methanol extract (BCG)																															
1.0 mg. + extraction residue, 0.3 mg., IP in distilled water	8	0	1	1	2	2	4	11	11					68	94	94	105	119	132	139	143					112	51	0.010
3.0 mg. + extraction residue, 0.9 mg., IP in distilled water	8	0	9	10	11	15	16	18	25					74	74	85	86	95	106	109	118					93	26	0.095
Distilled water, 1.5 ml., IP	8	0	0	0	0	0	0	0	1					44	50	56	70	80	94	97	102					74	—	—
Freund's adjuvant, 0.72 ml., IP	8	0	0	0	0	0	0	0	0					59	74	75	80	92	100	101	105					86	16	0.340
Shigella polysaccharide, 0.05 mg., IP in distilled water	8	0	0	0	0	0	0	0	0					58	59	64	81	87	91	91	96					78	5	0.399
Extraction residue, 0.9 mg., IP in distilled water	8	5	8	8	10	13	16	16	19					63	70	77	94	96	98	108	128					92	24	0.097
Living BCG§, 0.5 ml., SC	12	12	13	15	16	17	17	17	18	19	20	21	23	71	87	97	100	105	105	107	117	123	124	129	156	110	49	0.002

* Tuberculin skin test with 200 tuberculin units of PPD.

† IP = intraperitoneally.

‡ SC = subcutaneously.

§ Reconstituted suspension of a freshly obtained lyophilized preparation containing 6×10^6 living organisms per ml.

TABLE 3

POSTINFECTION SURVIVAL TIME OF GUINEA PIGS VACCINATED WITH METHANOL EXTRACT OR LIVING BCG

Vaccine	Days of Survival of Each Animal following Intramuscular Challenge Infection,* Two Months after Vaccination (8 Animals per Group)								Mean Survival Time	Per Cent Increase of Mean Survival Time over Distilled Water Controls	Statistical Significance of Comparisons of Survival Times of Vaccinated with Control Groups (Mann-Whitney Test)
									(*days*)		(P)
Methanol extract (BCG)											
3.0 mg., IP†, in distilled water	59	77	88	96	96	98	105	180	100	20	0.080
6.0 mg., IP, in distilled water	56	67	68	73	73	92	102	102	79	−5	0.480
Distilled water, 1.5 ml., IP	56	63	65	78	79	87	92	143	83	—	—
Living BCG‡, 0.5 ml., SC§	91	107	108	115	126	139	156	160	125	51	0.003

* Challenge infection with 0.5 ml. of a 1:1,000 dilution of a seven-day-old culture of the Branch strain of *M. tuberculosis*.

† IP = intraperitoneally.

‡ Reconstituted suspension of a freshly obtained lyophilized preparation containing 6×10^6 living organisms per ml.

§ SC = subcutaneously.

contracted infections almost always occur with quite small numbers of organisms. This assumption also appeared to be erroneous, however. As seen in table 3, for example, the resistance displayed by guinea pigs vaccinated with an identical methanol extract preparation and under the same conditions, as described in *Experiment 2*, but infected two months later with only one-tenth the number of virulent tubercle bacilli, was still of a low and statistically insignificant order. This finding, too, was not consistent, because on other occasions methanol extracts *did* prove effective when the size of the infecting inoculum was of the order shown in table 3. The number of tubercle bacilli contained in 0.5 ml. of even a 1:1,000 dilution of a luxuriant seven-day-old liquid culture is still, of course, very much greater than the number of organisms likely to infect a human being in nature, and methanol extracts might conceivably exhibit a more consistent immunogenic activity against really small infections. The problems of maintaining large numbers of animals for the long periods of time necessary to answer this question were, unfortunately, prohibitive. At any rate, even *were* methanol extracts to prove to be more consistently and more markedly active under such conditions, they would still have to be considered definitely less potent than the living vaccine. Alternately, the relatively high degree of protective activity displayed by methanol extract in *Experiment 1* might conceivably be ascribed to the fact that the challenge infection in that experiment was introduced by the intraperitoneal route. If the extract's activity were indeed limited to this circumstance, it would also have to be adjudged as valueless vis-a-vis living BCG for any practical prophylactic purpose, although still of considerable theoretic interest.

Still another possible explanation for the fluctuations in the immunogenic behavior of methanol extracts was suggested by the consideration that a vaccination procedure may, under certain circumstances, achieve an effect opposite from the one intended: a lowering of resistance. This might be brought about by different means.

First, it has been found (5) that killed tubercle bacilli and some of their fractions increase susceptibility to certain other infections when the period between vaccination and infection is short and can, under some circumstances, activate chronic into acute, heterologous infections. Although the vaccination-challenge interval in many of the present experiments in which methanol extracts were found to have only low protective activity was as long as two months, it must be considered not entirely improbable that this period of time was not quite long enough, in the case of the guinea pig, to escape any deleterious nonspecific effects which the preparations might evoke. The sporadic inefficacy of various vaccines could arise, according to this point of view, from the combined effects of the experimental tuberculous infection superimposed on an unrelated infection elicited or exacerbated by the vaccine. Such an infection alone, it was postulated, might have been withstood with little evidence of illness, but

have hastened the death of tuberculous animals; only a much more exacting histopathologic investigation than was here performed on the organs of animals that died after tuberculous challenge could have revealed the existence of such a situation.

Second, some of the reactions produced by a vaccine may *directly* lower resistance against the disease which it is meant specifically to prevent or ameliorate. The existence of a "negative" period of specific resistance after some vaccinations has been recognized for some time, and several possible mechanisms can be envisaged to account for this. Such a decrease of homologous resistance could well result from precisely the same course of events which causes a lowering of heterologous resistance—an impairment of the phagocytic function of the reticulo-endothelial cells, for instance. Immunologic factors of a more classic nature might also be involved. An example in point is the enhanced susceptibility to typhoid fever, the so-called "provocation" typhoid, which sometimes is a consequence of an antityphoid vaccination which has been given to an already infected individual, during the incubation period of the disease (51). Such a mishap is probably the result of the liberation of toxic quantities of typhoid endotoxin which is brought about by the rapid immunologic destruction of large numbers of the infecting bacilli, caused, in turn, by the heightened antibody response elicited by the vaccine. Conceivably, tubercle bacilli might also release certain of their components as a consequence of direct immunologic damage or of the inflammatory reaction evoked by them in tissues specifically hypersensitive or primed to respond with a Schwartzman-like reaction to their reintroduction. The substances released from the bacilli might be inherently toxic or toxic by virtue of the host's hypersensitivity. The effect on host resistance in either case could be pronouncedly inimical. The large numbers of tubercle bacilli employed in this study to infect animals by the intramuscular and intraperitoneal routes make the development of such a situation not entirely implausible.

Another basis for a directly inimical effect of a vaccine might be its induction of a temporary state of desensitization, or of immunologic paralysis, to antigens of the homologous, disease-causing organism, thus crippling any defense mechanisms based on antibody.

Thus, when all the possible deleterious effects which may grow out of the introduction of bacterial substances into the tissues of higher animals are taken into consideration, an effective vaccine must be looked upon as one whose specific and nonspecific resistance-stimulating properties exceed its propensities in the opposite direction. Whether the balance of the biologic reactions induced by a microbial vaccine will favor or defeat the development of a particular state of immunity is likely to depend not only on the qualities of the vaccine, but also on the circumstances of vaccination and infection. The various detrimental sequelae of vaccination which have been mentioned are most likely to be operative when infection by large numbers of organisms takes place soon after vaccination. How "soon" would be likely to depend on the nature of a vaccine and of the disease against which it is directed. In order to determine, therefore, whether the frequent immunogenic inefficacy of methanol extract could be attributed to a temporary overshadowing of its protective by its harmful activities, experiments were performed in which tuberculous challenge took place as late as five months after infection.

Late Post-Vaccination Challenge

The results of one such experiment are shown in table 4. Animals vaccinated with the same preparations described in *Experiment 2*, and appearing to be in good health when infected with virulent tubercle bacilli five months later, were found to be protected to a greater extent by living BCG than by any of the extract vaccines. Repeated intraperitoneal injections of extract suspended in water were ineffective. In other experiments, however, in which methanol extracts were administered and their protective activity tested under conditions thought to be very similar, the protection bestowed by them was occasionally found to be considerably greater.

It thus appeared that none of several of the more obvious variables in the techniques of administration and testing of methanol extract vaccines—strain origin, dose, route, and vehicle of the vaccine, time and size of the challenge infection—could be held clearly accountable for the differences in their protective efficacy encountered from one experiment to the next.

Experiments were then undertaken to determine whether variations in the degree of contami-

TABLE 4

POSTINFECTION SURVIVAL TIME OF GUINEA PIGS VACCINATED WITH METHANOL EXTRACT, METHANOL EXTRACT AND ADJUVANTS, OR LIVING BCG, AND INFECTED 5 MONTHS LATER

Vaccine	Days of Survival of Each Animal following Intramuscular Challenge Infection* (8 Animals per Group)								Mean Survival Time	Per Cent Increase of Mean Survival Time over Distilled Water Controls	Statistical Significance of Comparisons of Survival Times of Vaccinated with Control Groups (Mann-Whitney Test)
									(*days*)		(F)
Methanol extract (BCG)											
3.0 mg., IP† in distilled water	79	85	85	95	106	122	130	131	104	21	0.032
6.0 mg., IP in distilled water	60	75	85	95	97	106	114	118	94	9	0.164
1.0 mg., IP in 0.24 ml. Freund's adjuvant‡	80	86	100	104	117	122	126	145	110	28	0.025
Methanol extract (human)											
3.0 mg., IP in 0.72 ml. Freund's adjuvant§	89	91	92	95	101	104	118	119	101	17	0.032
Methanol extract (BCG)											
1.0 mg. + Shigella lipopolysaccharide, 0.05 mg., IP in distilled water	72	84	85	93	115	125	128	140	105	22	0.041
3.0 mg. + Shigella lipopolysaccharide, 0.05 mg., IP in distilled water	74	75	82	84	117	125	129	165	106	23	0.059
3 × @2.0 mg., IP in distilled water‖	P¶	24	72	85	89	94	119	134	88	2	0.215
Distilled water, 1.5 ml., IP	63	65	71	72	77	84	109	148	86	—	—
Living BCG, 0.5 ml. SC**, ††	83	97	97	114	129	130	163	193	126	47	0.010

* Challenge infection with 0.5 ml. of a 1:100 dilution of a six-day-old culture of the virulent Branch strain of *M. tuberculosis*.

† IP = intraperitoneally.

‡ Seven of the 8 animals in this group were found to be tuberculin sensitive when tested 58 days after vaccination.

§ Six of the 8 animals in this group were found to be tuberculin sensitive when tested 58 days after vaccination.

‖ The second vaccination was given 2 months after the first, and the third 6 weeks later.

¶ P = died previous to challenge, cause undetermined.

** SC = subcutaneously

†† Reconstituted suspension of a freshly obtained lyophilized preparation containing 6×10^6 living organisms per ml.

nation of methanol extracts with bacterial bodies or debris might be responsible for these fluctuations. Filtration of the hot methanolic extract solutions through Seitz filters did not remove the small quantities of weakly acid-fast bacterial debris which was occasionally seen in extract preparations, but filtration through "cella-filters" appeared to remove all traces of this material. In an experiment in which groups of 8 guinea pigs each were vaccinated intraperitoneally with 3.0 mg. (dry weight) quantities of filtered and unfiltered methanol extract suspended in Freund's incomplete adjuvant, all of the animals receiving the unfiltered material were found to be highly sensitive to 200 TU of PPD injected intradermally four weeks later, but none of the animals injected with the filtered extract reacted. These observations alone do not prove that the tuberculin-sensitizing powers of some lots of methanol extract were in fact due to contamination with particulate bacterial material, because some component of the extract itself may have had this property and conceivably have lost it as a consequence of physicochemical changes which occur not uncommonly when complex organic materials are passed through very fine filters. However, it was found in subsequent experiments that quantities as small as 0.01 mg. (dry weight) of a preparation of finely ground phenol-killed tubercle bacilli, consisting

partly of intact organisms and partly of particulate debris, were sufficient to elicit a weak, transient degree of tuberculin skin hypersensitivity in guinea pigs, even when the material was administered in aqueous, not in adjuvant, suspension (by the intraperitoneal route). It is quite likely, therefore, that filtration abolished the sensitizing powers of methanol extracts because it removed quantities of bacterial debris which, though small, may still have been in the range of allergenic capacity. Regardless of the reason for the destruction of allergenicity, the filtered, nonsensitizing extract preparations were found in further experiments to be no more or less predictable than the parent substance in eliciting heightened resistance to tuberculosis. When the filtered extracts *did* exhibit an immunogenic effect, it was essentially of the same order. Apparently, then, removal of its sensitizing activity did little to solve the problem of the immunogenic variability of this fraction.

Comment: These experiences with methanol extracts of tubercle bacilli and the similar results obtained by others with such preparations (52) seem to indicate that the unpredictability of their protective activities lies with the substances tested, rather than with the experimental circumstances of the tests. Many reasons might be advanced for this. Certain steps in the methods of preparation might destroy much of the extracts' activity. As the preparatory procedures have not been firmly standardized, it is not at all unlikely that relatively minor differences may produce very different end-products. A precedent for such an assumption comes from the many observations that *intact* killed tubercle bacilli are often very sensitive to certain chemical or physical manipulations (1). It also appears quite possible that many batches of methanol extract do not represent the entire, methanol-soluble moieties of the tubercle bacillus. The procedure of extraction here described did not extract the killed organisms exhaustively, and it may be that the less soluble fractions, which are obtained only by repeated, long extractions, represent the immunogenically more active portions. (It is of special interest to note in this connection that methanol-soluble fractions of tubercle bacilli obtained *after* the first 100 hours of continuous extraction at 55° C. are especially effective in bestowing nonspecific resistance on mice against infection by *P. pestis* (39).) The variability of methanol extract immunogenic activity towards homologous, i.e., tuberculous, infection might, accordingly, reflect, at least in part, variations in the length of time of extraction by which a particular lot of the material has been obtained or in the particle size of the starting bacillary mass and its consequent accessibility to the solvent.

Immunogeniciuy and Allergenicity of Methanol Extraction Residue and Combinations of Extract and Residue

It was seen in table 2 that the only circumstance under which the methanol extract preparation tested in that experiment provided an enhancement of resistance to tuberculous infection of the same order as accomplished by living BCG was when it was given together with a small quantity of the extraction residue. In previously reported immunizations of mice, the residues of methanolic extraction were also found to enhance the extract's activity (10, 46). It was decided, therefore, to test further the immunogenic activity of the residue and its adjuvant-like effects for methanol extracts not immunogenic by themselves.

Experiment 3: Groups of 20 guinea pigs each, consisting of equal numbers of males and females, were vaccinated with 1.0 and 3.0 mg. (dry weight) quantities of either filtered or nonfiltered, freshly prepared methanol extract preparations suspended in distilled water. Other groups of animals received either 0.3 or 0.9 mg. (dry weight) amounts of the residue of the extraction, or combinations of extract and residue. A control group was given an injection of 1.0 ml. of distilled water, and one group of 20 animals was vaccinated with 1.0 ml. of a 1:4-dilution of a six-day-old culture of the BCG-Phipps strain. All injections were given by the intraperitoneal route. Sixteen days later, 10 animals from each group (5 males and 5 females) were skin-tested by an intradermal injection of 0.2 ml. of a 1:10 dilution of the Weybridge mammalian Old Tuberculin, containing 2,000 TU. (A second tuberculin test, performed five weeks later, gave essentially similar results, except that the degree of skin hypersensitivity of some of the groups vaccinated with the nonliving preparations had begun to wane, while that of the BCG-immunized animals remained as great as at the earlier test.) Thirteen weeks after the first tuberculin test, i.e., fifteen weeks after vaccination, all animals were infected by an intramuscular injection of 0.5 ml. of a 1:100 dilution of a seven-day-old culture of the virulent Vallée strain of bovine tubercle bacilli; this inoculum contained 5×10^5 viable organisms. The results of the preinfection tuberculin test and the postinfection survival times of the animals are presented in table 5.

Vaccine (All vaccines administered intraperitoneally in distilled water, except the living BCG which was given subcutaneously)	Tuberculin Skin Hypersensitivity* 16 Days after Vaccination: Average Diameter of Induration at Site of Injection after 48 Hours (Average Reaction of 10 Animals)	Days of Survival of Each Animal following Intramuscular — Males										Mean Survival Time	Per Cent Increase†	Statistical Significance‡
	(*mm.*)											(*days*)		(P)
Methanol extract														
Unfiltered, 1.0 mg.	0	P§	40	44	47	49	57	98	102	106	148	77	−5	>0.1
Unfiltered, 3.0 mg.	2	P	39	45	69	73	74	77	91	107	145	80	−1	>0.1
Filtered, 1.0 mg.	0	40	41	42	61	87	105	124	128	142	175	95	17	>0.1
Filtered, 3.0 mg.	0	54	54	72	82	88	111	116	125	129	149	98	21	>0.1
Residue, 0.3 mg.	11	P	40	63	72	83	87	107	113	123	138	92	14	>0.1
Residue, 0.9 mg.	17	P	45	79	99	106	126	129	131	176	199	121	49	0.02<P<0.
Methanol extract														
Unfiltered, 1.0 mg. + residue, 0.3 mg.	13	41	64	83	101	110	123	126	139	153	156	110	36	0.05
Unfiltered, 3.0 mg. + residue, 0.3 mg.	15	P	44	106	106	110	124	131	148	148	151	119	47	0.002<P<
Unfiltered, 1.0 mg. + residue, 0.9 mg.	13	78	81	98	102	105	117	119	124	141	150	112	38	0.002<P<
Filtered, 1.0 mg. + residue, 0.3 mg.	14	54	67	86	92	97	104	132	138	166	211	115	42	0.05<P<0
Filtered, 1.0 mg. + residue, 0.9 mg.	17	71	98	105	115	129	141	152	153	173	218‖	126	56	0.002<P<
Distilled water, 1.0 ml.	0	54	61	71	78	81	81	86	91	101	101	81	—	—
Living BCG¶	27	113	114	133	134	139	142	144	147	151	165	138	70	0

* Tuberculin test with 2,000 TU of Old Tuberculin.

† Per cent increase of mean survival time over that of the distilled water control group.

‡ Statistical significance of comparisons of survival times of vaccinated groups with those of distilled water control group (Mann-Whi

§ Animal died previous to infection.

‖ Sacrificed in order to terminate experiment; not included in average.

¶ 1.0 ml. of a 1:4 dilution of a six-day-old Dubos-Tween medium culture.

POSTINFECTION SURVIVAL TIME OF GUINEA PIGS VACCINATED WITH METHANOL

Vaccine (All vaccines administered intraperitoneally in distilled water, except the living BCG which was given subcutaneously)	Days of Survival of Each Animal following Inhalation I — Males										Mean Survival Time	Per Cent Increase*	Statistical Significance
											(*days*)		(P)
Methanol extract													
Unfiltered, 10 mg.	24	25	27	27	29	29	35	37	40	64	34	31	0.02
Unfiltered, 3.0 mg.	P‡	P	P	P	P	26	29	31	32	42	32	23	<0.05
Filtered, 1.0 mg.	22	23	25	25	26	27	28	31	45	48	30	15	>0.1
Filtered, 3.0 mg.	20	22	23	25	25	27	28	31	39	81	32	23	>0.1
Residue, 0.3 mg.	22	23	32	33	37	44	54	84	91	104	52	100	0.02<P<0.
Residue, 0.9 mg.	P	31	39	55	62	69	73	74	91	101	66	154	<0.002
Methanol extract													
Unfiltered, 1.0 mg. + residue, 0.3 mg.	30	31	39	40	43	46	49	51	52	61	44	69	<0.002
Unfiltered, 3.0 mg. + residue, 0.3 mg.	26	28	31	34	37	37	40	42	45	63	38	46	0.002
Unfiltered, 1.0 mg. + residue, 0.9 mg.	P	23	27	33	34	36	37	37	41	81	39	50	0.002<P<0
Filtered, 1.0 mg. + residue, 0.3 mg.	P	21	30	34	38	39	42	51	54	61	41	58	0.002<P<0
Filtered, 1.0 mg. + residue, 0.9 mg.	29	29	38	38	39	50	64	64	83	85	52	100	<0.002
Distilled water, 1.0 ml.	20	21	22	23	23	24	26	27	28	43	26	—	—
Living BCG‖	P	P	39	47	52	54	60	60	83	86	60	131	<0.002

* Per cent increase of mean survival time over that of the distilled water control group.

† Statistical significance of comparisons of survival times of vaccinated groups with those of distilled water control group (Mann-Wh

‡ P = animal died previous to infection. In this experiment, a number of these animals were sacrificed upon appearing not well, to pr autopsy of animals dying *after* tuberculous infection.

§ Sacrificed in order to terminate experiment; not included in average.

‖ 1.0 ml. of a 1:4 dilution of a six-day-old Dubos-Tween medium culture.

E 5

TH METHANOL EXTRACT AND BACTERIAL RESIDUE, ALONE OR IN COMBINATION, OR WITH LIVING BCG

Challenge Infection 15 Weeks after Vaccination (20 Animals per Group)													Mean Survival Time of *Entire* Vaccination Group	Per Cent Increase of Mean Survival Time of Entire Vaccination Group over That of Distilled Water Controls	Statistical Significance of Comparisons of Entire Groups (Mann-Whitney Test)
Females										Mean Survival Time	Per Cent Increase†	Statistical Significance‡			
										(*days*)		(P)	(*days*)		(P)
2	44	61	72	81	84	102	105	106	147	84	14	>0.1	81	4	>0.1
P	37	42	46	47	48	50	53	76	112	57	−22	0.1	69	−12	>0.1
3	40	49	54	54	61	84	97	102	106	68	−8	>0.1	82	5	>0.1
5	56	61	64	78	82	84	126	140	156	88	19	>0.1	93	19	>0.1
3	38	60	94	94	100	122	136	141	149	97	31	>0.1	95	22	>0.1
P	P	58	81	121	136	146	158	163	164	128	73	0.012	125	60	<0.002
P	35	83	97	136	144	148	150	153	186	126	70	0.02	118	51	<0.002
P	61	87	100	103	136	137	144	208	218‖	122	65	0.014	121	55	<0.002
P	45	67	110	126	132	136	138	154	218‖	114	54	0.019	113	45	<0.002
0	71	72	89	99	126	144	157	173	178	118	59	0.02	117	49	0.002<P<0.02
P	43	65	98	105	131	135	136	148	167	114	54	<0.05	120	54	<0.002
P	P	24	57	59	64	64	81	122	124	74	—	—	78	—	—
7	90	99	126	129	132	132	144	169	218‖	122	65	0.002<P<0.02	130	67	<0.002

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E 6

CT AND BACTERIAL RESIDUE, ALONE OR IN COMBINATION, OR WITH LIVING BCG

n 15 Weeks after Vaccination (20 Animals per Group)													Mean Survival Time of *Entire* Vaccination Group	Per Cent Increase of Mean Survival Time of Entire Vaccination Group over That of Distilled Water Controls	Statistical Significance of Comparisons of Entire Groups (Mann-Whitney Test)
Females										Mean Survival Time	Per Cent Increase*	Statistical Significance†			
										(*days*)		(P)	(*days*)		(P)
	26	26	28	28	30	32	32	33	35	30	3	>0.1	32	14	0.002<P<0.02
	25	26	28	29	29	31	31	40	43	31	7	>0.1	32	14	0.02<P<0.05
	25	25	26	29	29	29	30	30	35	29	0	>0.1	30	7	>0.1
	23	24	28	28	31	32	32	39	49	31	7	>0.1	32	14	>0.1
	28	29	34	42	57	58	61	64	67	49	69	0.002<P<0.02	51	82	<0.002
	P	28	31	32	34	37	40	41	72	39	34	0.02<P<0.05	53	89	<0.002
	29	33	41	55	64	64	64	64	103	55	90	0.002<P<0.02	50	79	<0.002
	35	41	47	57	63	64	74	98	104	62	114	<0.002	50	79	<0.002
	33	34	34	37	50	56	57	65	81	48	65	0.002<P<0.02	44	57	<0.002
	29	32	37	40	46	52	55	72	218§	43	48	0.002<P<0.02	42	50	<0.002
	24	34	35	36	39	39	39	44	51	38	31	0.02	45	61	<0.002
	23	24	24	25	26	32	32	35	40	29	—	—	28	—	—
	37	45	45	45	47	48	57	60	64	50	72	<0.002	55	96	<0.002

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possibility of an outbreak of pseudotuberculosis which attacked animals in an adjoining room; no case of pseudotuberculosis was seen at

It may be seen in table 5 that neither the filtered nor the unfiltered methanol extract preparation here tested afforded by itself a significant degree of survival protection. The larger quantity of the extraction residue, 0.9 mg., was as effective in prolonging life as the living BCG vaccine; the smaller dose, 0.3 mg., provided no protection. When, however, this small quantity of residue was administered together with methanol extract, a degree of survival protection approaching that bestowed by the living vaccine was attained. The residue and residue-extract combinations sensitized most of the animals to tuberculin, but to a considerably lower degree than did the living BCG.

The survival times of male and female animals were, on the whole, very similar, both in the control and in the vaccinated groups. In the few instances in which there appeared to be a difference between males and females in the degree of statistically significant, i.e., $P = 0.05$ or < 0.05, survival prolongation vis-a-vis their respective sex controls, the females seemed to be protected to a somewhat greater extent.

Experiment 4: Groups of 20 guinea pigs each, consisting of equal numbers of males and females, were vaccinated with the same preparations described in the previous experiment. Fifteen weeks later, the animals were infected by *inhalation* of a cloud of tubercle bacilli of the bovine strain Vallée, giving an estimated lung retention (4) of 3,750 organisms. The day of death of each animal after infection and the increases in mean survival time over that of the distilled water control group are shown in table 6.

As may be seen from a comparison of tables 5 and 6, the control as well as the vaccinated animals succumbed much more rapidly to experimental tuberculosis established by the respiratory than by the intramuscular route, even though the number of infecting organisms introduced by the latter was more than 100 times greater. However, the *relative increases* in survival time bestowed by the vaccines were at least as great when infection was produced by inhalation and, indeed, in several instances protection was evident only when challenge infection was performed by this means. Thus, unfiltered methanol extract given alone provided a low but statistically significant measure of survival protection in *Experiment 4* but not in *Experiment 3*, and the smaller of the two quantities of residue assayed in both experiments, 0.3 mg., was efficacious when the animals were infected by inhalation but not when they were challenged by injection of virulent tubercle bacilli. (The same observation, that respiratory infection is a better means for revealing vaccination-induced resistance to tuberculosis in the guinea pig than is intramuscular injection, was also made in the previous study, in which animals were vaccinated with intact killed tubercle bacilli (4).) The combined extract-residue preparations were no more effective than either quantity of residue alone.

As in the preceding experiment, in which infection was given intramuscularly, the survival times of the nonvaccinated male and female controls challenged by inhalation were almost identical. Unlike the former experiment, however, there appeared to be appreciable sex differences in resistance among the immunized animals. As shown in table 6, males fared better than females in most instances in which there was a difference in significant survival prolongation relative to the nonvaccinated controls of each sex. However, the apparent female superiority noted in one or two vaccination groups precludes the generalization that the male guinea pig is consistently more capable of developing acquired immunity to tuberculosis (of bronchopulmonary origin), a situation suggested by the observations in mice of Hoyt and his colleagues (53).

Toxigenicity of Methanol Extracts and Extraction Residues

Both the methanol extract and the extraction residue preparations studied in these experiments appeared to be entirely innocuous in the quantities employed. The animals did not lose weight or display any other gross symptoms of illness or discomfort after vaccination. The extract did not usually cause the formation of visceral adhesions after intraperitoneal administration, and the residue did so to a lesser extent than equal quantities of intact, phenol-killed tubercle bacilli (4).

Discussion

These studies provide further evidence that a degree of enhanced resistance to tuberculosis can be elicited not only by living or by killed intact tubercle bacilli, but also by some of their isolated components. Of the two substances tested, a methanol-soluble extract and the residue of extraction, the former exhibited immunogenic

activity only sporadically and then generally of a lesser order than living BCG. The latter appeared, in several large experiments conducted under different experimental conditions, to be as effective a vaccine as the standard living preparation.

The frequently low and unpredictable immunogenicity of methanol extract came as a disappointment in view of its previously observed successes in immunizing mice (10, 45), and points, once again, to the risks of anticipating the biologic activity of a substance in one species on the basis of its action in another. If man resembles guinea pig more than mouse in his responses to antituberculosis vaccines, crude methanol extracts would appear to be of little value in preventive medicine. Certainly, methanol does not emerge as the solvent of choice for obtaining immunogenic fractions from tubercle bacilli. In part, the alcohol may destroy some of the protective activity of the moieties which it dissolves, but it also does not seem to remove readily more than a segment of the immunogenically active components of the organism. The assumption might be made that in matters of immunogenicity the sum of its parts can be greater than the whole, because the removal of certain immunogenically deleterious parts of an organism, for example some of the lipids of the tubercle bacillus, may permit much greater activity of the remaining portions. The high degree of protective activity of methanol extraction residues might then reflect a heightening of potency due to removal of undesirable fractions, rather than a retention of most of the intact organism's immunizing components; the low immunogenicity of the extract, on the other hand, could be due to its content of such undesirable materials rather than to an intrinsic incapacity to protect. However, the absence of any readily discernible toxic activity of the extract and its inactivity under a variety of test conditions, even those most likely to de-emphasize the role of any immunogenically unfavorable component which it may contain, makes it improbable that this explanation can account for more than a small part of the truth.

Yet, perhaps more important than the apparent failure of crude methanol extract as a practical prophylactic agent in the guinea pig is its contribution to the resolution of one of the more controversial issues in tuberculosis studies: the role of tuberculin skin hypersensitivity in protective immunity. In those experiments in which the extract *was* capable of increasing the resistance of the animals to tuberculosis, it usually did so without making them skin-hypersensitive to Old Tuberculin or purified protein derivative. Indeed, the quantities of unfiltered extract necessary to elicit tuberculin hypersensitivity were almost invariably in excess of the immunogenically active amounts. It must be pointed out that absence of *skin* hypersensitivity to an antigen does not always indicate total absence of *systemic* hypersensitivity. Moreover, the tuberculin preparations here employed to test for skin reactivity were the usual heated preparations, and absence of a reaction to these does not necessarily imply absence of hypersensitivity to unheated tuberculoproteins. The lack of any correlation between the skin-sensitizing and immunizing activities of methanol extract may therefore still leave moot the question of the role of anti-protein allergy in tuberculosis immunity, but it does at least provide further evidence that the loss of the tuberculin test should not be accepted as an unavoidable consequence of effective immunization.

The independence of skin hypersensitivity to tuberculin and acquired resistance to tuberculosis was also indicated by the observation that vaccination with extraction residue was as protective as vaccination with living BCG, but evoked a much lower degree of sensitivity.

This and the preceding study (4) thus support the contentions of many other investigators (1) that the development of acquired immunity to tuberculosis does not require infection with living tubercle bacilli, but can be bestowed to at least an equal degree by components of the bacilli which are nonliving, nontoxic, and probably also non-skin-sensitizing. In view of the growing recognition that certain fractions of microorganisms can initiate resistance-lowering as well as resistance-enhancing reactions in higher animals, and that even a primarily nonspecific effect may influence specific immunity, it appears not unlikely that isolated fractions of the bacillus may eventually prove to be superior vaccinating agents.

It is also quite clear, on the other hand, that the development of a highly effective fraction-vaccine still requires considerable effort. The variations from laboratory to laboratory in the conditions under which nonliving antituberculosis vaccines have been tested, the chemical heterogeneity of the substances studied, and the

chronic nature of the disease and consequent difficulties of evaluating a vaccine have prevented the establishment of universal criteria and standards by which the preparation and assay of such vaccines can be governed. The choice of methods has therefore remained a largely haphazard one and, naturally, so have the results. Indeed, the state of anarchy in this field of investigation is so great that a meaningful comparison of experiences and the establishment of the real value of newly described preparations is often exceedingly difficult, and the chances of gaining general approbation and endorsement of a vaccine for human use very remote.

The imperative requirement for improvement of this situation lies with a systematic, comparative study of the immunologic and chemical properties of a large number of fractions of tubercle bacilli derived from several different strains, and grown and killed by a variety of different procedures. Such a study might provide two sets of information: the conditions most favorable for the production of highly effective vaccines and some criteria of efficacy—possibly serologic—other than survival tests in animals, so that the individual investigator is not faced with the insurmountable difficulty of having to subject each minor procedural variation to such an assay. The very large financial demands of such a study could undoubtedly be met best by a laboratory specifically dedicated to this purpose and equipped not only to initiate anew a comprehensive study of the immunogenic behavior of tubercle bacillus fractions, but also prepared to subject to comparative tests fractions already investigated and recommended as effective. If this undertaking appears—and rightly so—to be a very large one, it must be remembered, as encouragement and incentive, that the principle of the immunogenic efficacy of nonliving tubercle bacilli and some of their fractions is already firmly established and that the task still faced is limited to the discovery of the *most* desirable preparation.

Summary

The immunogenic activity of two fractions of phenol-killed, acetone-washed tubercle bacilli was investigated in guinea pigs, employing post-infection survival time as the criterion of efficacy.

A crude methanol extract was found capable of bestowing, on occasion, a degree of enhanced resistance against subsequent infection with living, virulent tubercle bacilli. Vaccination with immunogenically effective quantities of the extract did not usually result in the development of tuberculin skin hypersensitivity. The degree of survival protection elicited by the extract was often lower than that given by living BCG, however, and could frequently not be demonstrated at all. Possible reasons for the sporadic nature of the immunogenic behavior of this preparation are discussed.

In contrast, the immunogenic powers of residues of methanol extractions were found to be consistently of an order approaching that of the living BCG vaccine. The residues produced a low degree of tuberculin skin hypersensitivity.

Combinations of immunogenically inactive preparations of methanol extract and quantities of their residues too small to elicit protection by themselves appeared to be synergistic.

The future approach to the development of an effective and acceptable antituberculosis vaccine is discussed in the light of these findings and with regard to recent developments in the area of the immunologic nonspecificity of the effects initiated by microorganisms in the tissues of higher animals.

Sumario

Vacunación Anti-Tuberculosa con Vacunas No Vivientes. III. Vacunación de Cobayos con Fracciones de Bacilos Tuberculosos Matados con Fenol

Se investigó en los cobayos la actividad inmunógena de dos fracciones de bacilos tuberculosos matados con fenol y lavados con acetona, empleando como norma de la eficacia el tiempo de sobrevivencia posterior a la infección.

Un extracto bruto de metanol se mostró capaz de otorgar, a veces, cierta resistencia acrecentada contra la infección subsiguiente por bacilos tuberculosos virulentos, vivos. La vacunación con cantidades inmunogénicamente eficaces del extracto no dió habitualmente por resultado la formación de hipersensibilidad cutánea a la tuberculina. Sin embargo, la intensidad de la protección de la sobrevivencia incitada por el extracto fué a menudo menor que la proporcionada por BCG vivos y frecuentemente no podía observarse. Se discuten posibles razones de la naturaleza esporádica del comportamiento inmunógeno de esta preparación.

En contraposición, las facultades inmunógenas de los residuos de las extracciones con metanol se

mostraron constantemente de tenor que se aproximaba al de la vacuna de BCG vivos. Los residuos produjeron hipersensibilidad cutánea a la tuberculina de orden bajo.

Parecieron ser sinérgicas las combinaciones de preparaciones inmunogénicamente inactivas de extractos metanólicos y de cantidades de sus residuos que eran demasiado pequeñas para incitar protección por sí solas.

Discútese a la luz de estos hallazgos la senda futura que conducirá a la elaboración de una vacuna antituberculosa eficaz y aceptable y en relación con recientes desenvolvimientos en la zona de la anespecificidad inmunológica de los efectos iniciados por microbios en los tejidos de animales superiores.

Resume

Vaccination contre la tuberculose par des vaccins tués. III. Vaccination de cobayes avec des fractions de bacilles tuberculeux tués par du phénol

L'activité immunisante de deux fractions de bacilles tuberculeux, tués au phénol et lavés à l'acétone, fut étudiée chez des cobayes, en se servant du temps de survie après l'infection comme criterium d'efficacité.

On trouva qu'un extrait de méthanol brut peut accroitre dans certaines occasions, le degré de résistance contre une infection ultérieure par des bacilles tuberculeux vivants, virulents. La vaccination avec des quantités donnant une immunité effective ne produit pas ordinairement le développement d'hypersensibilité de la peau, à la tuberculine. Le degré de protection persistante apportée par l'extrait fut souvent inférieur à celui donné par le BCG vivant, cependant, il fut souvent impossible de le démontrer. Les auteurs discutent les raisons possibles de la nature sporadique du comportement immunisant de cette préparation.

Au contraire, on trouva que les pouvoirs immunisants des résidus des extractions du méthanol approchaient régulièrement ceux du BCG vivant. Les résidus produisirent un faible degré d'hypersensibilité de la peau à la tuberculine.

Il semble qu'il y ait une action synergique de la part des combinaisons de préparations inactives d'extrait de méthanol et de leurs résidus, en quantités insuffisantes pour établir par eux-mêmes une protection.

A la lumière de ces données, les auteurs discutent les considérations futures sur la mise au point d'un vaccin antituberculeux efficace et acceptable, et considèrent en même temps les acquisitions réçentes dans le domaine du manque de spécificité immunologique des effets produits par des microorganismes dans les tissus des animaux les plus hauts dans l'échelle animale.

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TUBERCULOSIS DISSEMINATORS[1,2]

A Study of the Variability of Aerial Infectivity of Tuberculous Patients

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INTRODUCTION

Since October, 1956, a quantitative study of air-borne infection has been in progress at the Veterans Administration Hospital in Baltimore. The frequency of occurrence of tuberculous infections in guinea pigs exposed to air exhausted from a six-bed ward of patients with active pulmonary tuberculosis has been determined. The experimental unit is an isolated ward of six beds, with a controlled ventilating system; a constant quantity of air is continuously removed from the ward and, just before being exhausted from the hospital, is passed through a large animal exposure chamber. Riley and associates have reported the details of the method (1–3).

It was not anticipated that the detailed bacteriology involved would be of special interest. However, in the course of the study it became evident that a very few of the many participating patients were causing these infections and that the majority of the animal infections were caused by drug-resistant bacilli. This paper is a retrospective analysis of these findings.

METHODS

Patients: The finding of acid-fast bacilli on a smear of 72-hour sputum concentrate was set as the criterion for admission of patients to the ward. During the first seven to eight months the ward was predominantly occupied by patients who were treatment failures, with tubercle bacilli consistently demonstrable in the sputum. By May, 1957, difficulty was encountered in obtaining such patients; therefore, from May to September, 1957, only previously untreated cases of tuberculosis were used. The patients were admitted to the ward as soon as the diagnosis of tuberculosis was confirmed by finding tubercle bacilli in the sputum. While on the ward they received antimicrobial therapy. During the period when these patients with new cases of tuberculosis occupied the ward, the animal infections stopped. Therefore, in the autumn of 1957, the predominant patient population was again changed to patients who were treatment failures and were consistently discharging tubercle bacilli in the sputum. Some of the patients with drug-resistant bacilli harbored organisms resistant to all of the drugs with which they were being treated, while others were being treated with drugs which were believed to be effective; in some instances these included the so-called minor drugs.

Almost all of the patients had cavitary tuberculosis. The patients with the most extensive involvement and the largest numbers of acid-fast bacilli on microscopy of sputum were chosen in preference to others. Sputum smears were examined at monthly intervals until December, 1957; subsequent examinations were done every two weeks. Sputum was cultured monthly, and the drug susceptibilities of the strains of tubercle bacilli were determined about every three months.

In the previously untreated cases, the numbers of acid-fast bacilli demonstrable microscopically diminished at the successive monthly examination. Whenever it was anticipated that bacilli would no longer be demonstrable by microscopy of sputum, the patient was removed from the study. The patients with drug-resistant bacilli and re-treatment cases with drug-susceptible bacilli more consistently discharged the same number of tubercle bacilli into the sputum.

Estimates of the number of days each patient had sputum containing tubercle bacilli demonstrable by microscopy and the crude estimation of the quantity of bacilli (Gaffky count) were made by assuming that any change in the findings occurred midway between examinations. Similar estimates were made for culture results. The values found are presented in table 1. Not included in the total number of patient-days are the days of occupancy by patients with sputum consistently negative for *M. tuberculosis* on both direct microscopy and culture (patients represented by *N* in figure 2). The total number of patient-days of occupancy by patients harboring drug-resistant and drug-susceptible bacilli (*see* figure 2) were about equal as determined by examination on microscopy and culture. However, patients with drug-susceptible bacilli had fewer days in which examination showed large numbers of acid-fast bacilli on microscopy and three and four plus growth on culture. Two patients (*WI* and *RU*) contributed a total of 704 days (31 per cent) of occupancy by patients with drug-resistant bacilli.

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TABLE 1

RESULTS OF EXAMINATION BY MICROSCOPY AND CULTURE OF SPUTUM OF PATIENTS SHOWN IN FIGURE 2

A. Results of Sputum Examination by Microscopy

Patient Group	Total Patient Days	Days Negative for *M. Tuberculosis*	Days Positive for *M. Tuberculosis*	Gaffky I–III	Gaffky IV–VIII
Drug-susceptible bacilli	1,985	601	1,384	909	475
Drug-resistant bacilli*	2,246	331	1,915	1,177	738
	(1,542)	(278)	(1,264)	(692)	(572)

B. Results of Sputum Cultures

Patient Group	Total Patient Days	Days Cultures Positive for *M. Tuberculosis*	Culture 3 to 4+	Culture 1 to 2+	Culture 0–50 Colonies	Culture Result Unknown
Drug-susceptible bacilli	1,985	1,869	654	905	310	0
Drug-resistant bacilli*	2,246	2,208	1,575	510	81	42
	(1,542)	(1,519)	(914)	(482)	(81)	(42)

* The figures obtained after excluding patients *RU* and *WI* are given in parentheses.

In table 1, the results of sputum microscopy and culture in the drug-resistant group minus the contribution of these 2 patients are presented in parentheses.

Guinea pigs: Guinea pigs aged six weeks were quarantined for three to four weeks. They were then tuberculin-tested by injection intradermally of 0.002 mg. of PPD. Reactions were read at twenty-four and forty-eight hours. No positive reactions were encountered among the quarantined animals. After this initial test they were admitted to the chamber and subsequently were tuberculin-tested at monthly intervals. A reaction was considered positive if there was easily palpable induration greater than 10 mm. in diameter with necrosis. Animals showing such reaction were removed from the chamber and were autopsied within one to three days.

Bacteriology: The drug-susceptibility tests were carried out in the clinical laboratory until September, 1958. At that time the handling of all material was transferred to the recently opened microbiology laboratory of the research department. At this time a change was made in drug concentrations used in testing, and dilutions of cultured bacilli through 10^3 were used in testing drug resistance.

Both laboratories used the methods outlined in the Laboratory Manual of the Veterans Administration–Armed Forces Cooperative Study on the Chemotherapy of Tuberculosis (4).

The results of the drug-susceptibility testing given here are results of subculture of originally isolated bacilli. Growth in all control tubes was 4-plus. Growth was read as:

Negative – no growth
Colonies – less than 50 colonies
1+ – 50 to 200 colonies
2+ – more than 200 colonies
3+ – heavy growth; not confluent
4+ – confluent growth

RESULTS

The results of 41 guinea pig infections occurring from November, 1956, to April, 1958, have been previously reported (3). Of these 41 infections, 22 were cultured. It was demonstrated that 2 patients (*AR* and *JO*) of a total of 62 participating patients had caused 19 of 22 cultured guinea pig infections. This paper adds the results through November, 1958, which include 30 more infections, 26 of which were successfully cultured.

The number of animals exposed, the number infected, and the number of infected animals whose organisms were cultured are shown in figure 1. The present paper deals with the latter group of infections.

The time of residence of the patients, the time of infection of the guinea pigs, and the results of drug-resistance testing of the bacilli from patients and animals are shown in figure 2. Drug resistance was considered as any growth of tubercle bacilli in concentrations of 10 γ per ml. for streptomycin, 5 γ per ml. for isoniazid, and 10 γ per ml. for PAS. This roughly divided patients into a group in which isoniazid, streptomycin, or PAS would be expected to be effective and a group in which

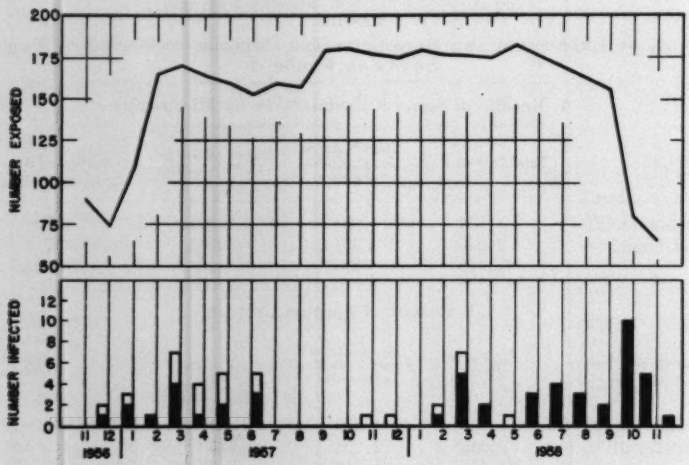

FIG. 1. Number of animals exposed related to number infected. Shaded area of column indicates number of animals from which bacilli isolated were tested for drug resistance.

they probably would not be effective. Of the former group, 9 patients were re-treatment cases (*WA*, *KE*, *DO*, *CO*, *HU*, *SI*, *NE*, *HI*, *KE*). The guinea pig infections are shown by the circles. The circles are so placed and are of such size as to cover an area corresponding to the two-week time interval during which the animal probably became infected, i.e., four to six weeks prior to the time the animal was found to be tuberculin positive. It should be noted that, if the infected animal was tested three weeks after infection, it might have been missed. In such case, it would not be found until infection had been present seven weeks. Therefore, these circles may fall short by one week of the maximal possible time between animal infection and the discovery of the presence of tuberculin sensitivity.

Several important findings emerge from figure 2. The time during which patients harboring drug-susceptible organisms were present (and receiving chemotherapy) on the ward is greater than that for which patients with resistant organisms were present. Two infections with drug-susceptible organisms occurred over the entire period. During the period June to September, 1957, when only patients with susceptible organisms were present, no guinea pig infections occurred.

The drug-susceptibility patterns of the organisms recovered from the guinea pigs showed variations and corresponded closely with those of organisms excreted by patients present in the ward at the probable time of infection. This suggested the possibility that detailed examination of the resistance patterns of patient and guinea pig organisms might reveal the actual patient sources of each infection. To do this would require the following: (*1*) Bacilli from the patient's sputum would have to show a resistance pattern similar to that of bacilli cultured from the guinea pig; (*2*) the infections would have to have started when the patient arrived on the ward and to have stopped when he left the ward; (*3*) he would have to be the only patient harboring organisms with the specific drug resistance under consideration who was present in the ward during this time.

On this basis the animal infections have been divided into nine groups. It is believed that one patient caused each group of these infections, with the exception of those of *Group VI*.

Since the grouping of infections and the relating of them to patients depend upon the matching of drug-resistance results, the comparability to be expected from repeated drug-resistance testing of the same bacteriologic specimen merits some consideration.

In discussing streptomycin-susceptibility testing, the American Trudeau Society Laboratory Subcommittee reported:

> In spite of some variation in the results of multiple *in vitro* determination of strepto-

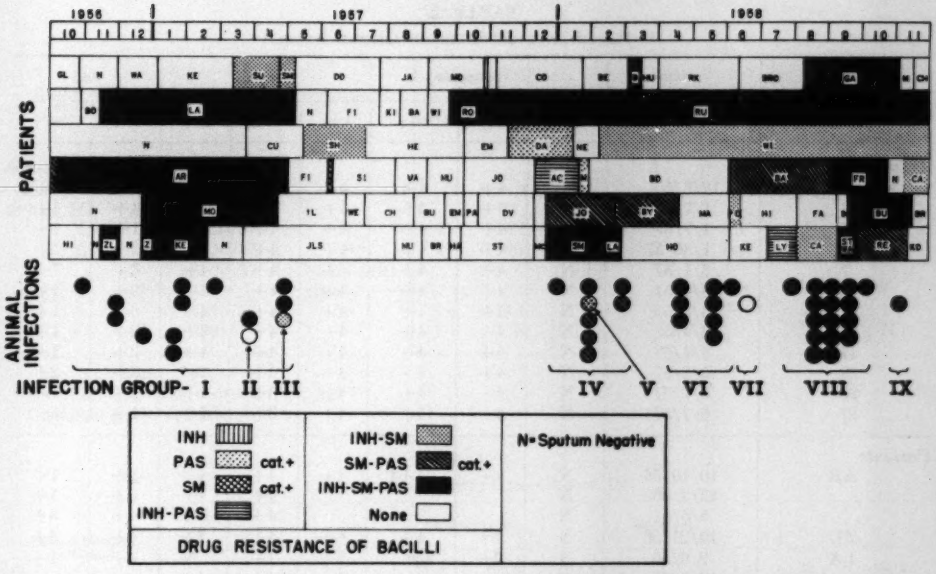

FIG. 2. Bars indicate time of residence of patients. Circles indicate date of infection of animals from which isolated bacilli were tested for drug resistance. Circles and bars are appropriately shaded to indicate drug resistance of bacilli. Roman numerals indicate infection group (*see* text). N refers to patients in residence who were negative by both direct microscopy and culture of sputum. SM = streptomycin; INH = isoniazid.

mycin sensitivity from the same specimen it is probable that duplicate tests in the same or in different laboratories will agree closely for the very sensitive and the very resistant organisms. Greater differences may be expected with organisms in the intermediate range. (5).

The two variables which are most difficult to control are errors in sampling and variations in inoculum size (4). The problem in sampling starts with the collection of sputum, and can be only partially obviated by the bacteriologist's sampling from all colonies.

In the method used here, inoculum size was controlled by turbidimetric standardization, which is far from precise. Slight variation in inoculum size can easily lead to a difference of one grade in growth. This is particularly true if the population is heterogeneous. Variation in inoculum size is particularly likely to affect results of PAS-resistance studies (6–8). It has been suggested that laboratory strains of PAS-resistant bacilli tend to be heterogeneous until there is associated isoniazid resistance (9). In the matching of results of drug-resistance testing of bacilli from the patients and from the guinea pigs in the data to be presented, it will be noted that greater variability has been allowed in PAS-resistance results than with streptomycin and isoniazid, that great consistency is evident when organisms have shown high degrees of resistance, and that less consistency of results is present with lower degrees of drug resistance. Nevertheless, variation is infrequently more than one grade on either side of the average growth in each drug concentration used.

The data in table 2 deal with the 12 infections of *Group I*. The complete results of drug-resistance testing of the animals' and patients' bacilli are given in this table. The important point to note here is that *all* of the animals' bacilli showed 4-plus growth in 100 γ of streptomycin, whereas only patients *AR* and *ZL* harbored such bacilli. Patient *ZL* was on the ward only two weeks. This group of 12 infections had its onset before *ZL* arrived and continued after he left. However, patient *AR* entered the ward approximately two weeks before the first

TABLE 2
RESISTANCE RESULTS IN GROUP I

Animal Number or Patient	Culture Date	Catalase	Streptomycin		Isoniazid			PAS	
			10 γ	100 γ	1.0 γ	5.0 γ	25 γ	10 γ	100 γ
Animals									
1	12/6/56	N	4+	4+	4+	4+		4+	2+
3	1/7/57	N	4+	4+	4+	4+		4+	1+
5	1/7/57	N	4+	4+	4+	4+		1+	1+
6	1/30/57	N	4+	4+	4+	4+		3+	1+
7	3/1/57	N	4+	4+	4+	4+	4+	3+	1+
8	3/1/57	N	4+	4+	4+	4+	4+	3+	1+
11	3/4/57	N	4+	4+	4+	4+	4+	3+	1+
12	3/4/57	N	4+	4+	4+	4+	3+	3+	1+
17	4/1/57	N	4+	4+	4+	4+	4+	3+	1+
23	5/8/57	N	4+	4+	4+	4+	2c*	3+	2+
25	6/7/57	N	4+	4+	4+	3+	1+	2+	1+
27	6/7/57	N	4+	4+	4+	3+	2+	1+	15c
Patients									
AR	10/10/56	N	4+	4+†	4+	4+	4+	2+	1+
	12/3/56	N				4+	4+	3+	1+
	5/6/57	N				4+	4+	4+	3+
ZL	10/21/56	N	4+	4+	4+	4+	3+	4+	3+
LA	2/6/56	N	2+	25c	2+	1+	N	4+	1+
	12/3/56	N			4+	4+	4+		
	8/12/57	N	4+	N		4+	4+	3+	1+
MO	12/19/57	N	3+	N	4+	4+	4+	N	N
	4/1/57	N	N	N		4+	4+	3+	1+
Z	11/20/56	N	1+	N		4+	4+	3+	1+
KE	12/17/56	N	3+	N	4+	4+	4+	4+	4+

* c = colonies.
† Cultures of 2 and 8/54 showed 3 and 4+ growth in 100 γ of streptomycin.
Catalase negative indicated by N
Catalase positive indicated by +

animal infection occurred, during his seven months' residence infections continued, and the last one appeared approximately one week before his transfer. Therefore patient *AR* is considered to be the source of these 12 infections.

The details of the next two groups are shown in table 3. *Group II* is infection No. 24, a completely suceptible bacillus which probably came from *KE*, *CU*, or *JLS*.

Group III is infection No. 29. Patients *SM* and *SU* are considered as possible sources. The need for matching of 4-plus growth of the bacilli from patient and animal in 100 γ of streptomycin is considered as eliminating patient *SU*. The difference in catalase activity between *SM* and infection No. 29 is attributed to heterogeneity of patient *SM*'s bacterial population which resulted in complete loss of catalase activity by July 15, 1958. It is pertinent to this point that a culture is considered catalase negative by the laboratory only if there is complete absence of bubbling with hydrogen peroxide.

Group IV (table 4) includes data on seven infections with catalase-positive streptomycin-PAS–resistant bacilli which occurred in December 1957, and in January and February, 1958. Prior to *JO*'s admission to the ward no patient with such bacilli had participated in the study. The infections started shortly after *JO*'s arrival and stopped when he left the ward. He was considered to be a second particularly hazardous source of infection.

So far as time relationships are concerned, patient *BY* might have caused infections Nos. 45 and 46. However, his organisms' drug resistance, as shown in detail in table 4, is only poorly in agreement.

Detailed results for *Groups V, VI, and VII* are shown in table 5. *Group V* is infection No. 40. This organism was resistant only to PAS. Patients

TABLE 3

RESISTANCE RESULTS IN GROUPS II AND III

Animal Number or Patient	Culture Date	Catalase	Streptomycin		Isoniazid			PAS	
			10 γ	100 γ	1.0 γ	5.0 γ	25 γ	10 γ	100 γ
Group II									
24	5/8/57	+	N	N	N	N	N	N	N
Group III									
29	6/10/57	N	4+	4+	4+	4+	4+	N	N
SM	4/16/57	+	4+	4+	3+	2+	5c*	N	N
	5/20/57	Weakly positive			4+	4+	4+		
	7/15/57	N	4+	4+	4+	4+	3+	2+	1+
SU	3/6/57	+	1+	N	15c	N		N	N

* c = colonies
Catalase negative indicated by N
Catalase positive indicated by +

TABLE 4

RESISTANCE RESULTS IN GROUP IV

Animal Number or Patient	Culture Date	Catalase	Streptomycin		Isoniazid			PAS	
			10 γ	100 γ	0.1 γ	1.0 γ	5 γ	10 γ	100 γ
36	2/6/58	+	4+	N	4+	3c*	N	2+	1+
39	3/5/58	+	4+	N	4+	1+	N	3+	1+
41	3/6/58	+	4+	N	4+	N	N	3+	2+
42	3/6/58	+	4+	10c	4+	4c	N	3+	25c
43	3/6/58	+	4+	N	4+	10c	N	3+	1+
45	4/3/58	+	3+	N	4+	10c	N	2+	10c
46	4/3/58	+	4+	N	4+	2+	N	2+	15c
JO	1/21/57	+	3+	N	4+	1+	N		
	10/14/57	+			4+	1+	N	3+	1+
	1/14/58	+			4+	1+	N		
	5/12/58	+			4+	2+	1+		
	9/8/58	+	4+	N	4+	2+	1+	3+	1+
BY	12/22/58	+	4+	3+	N	N		1+	N
	3/25/58	+			10c	N		N	N
	6/18/58	+	4+	4+	N	N		1c	

* c = colonies
Catalase negative indicated by N
Catalase positive indicated by +

DA and *MA* had such bacilli. In order to attribute this infection to *DA*, a time interval of seven weeks between infection and discovery of tuberculin sensitivity would have had to have existed. Patient *DA* showed 4-plus growth on routine culture and clinically showed continuing spread of disease despite streptomycin-isoniazid-PAS. He left the hospital against advice in March, 1958, and died one month later. Patient *MA* was in the ward five days; his culture for many months had shown only a few colonies. Either of these patients could have been the source of infection No. 40.

Group VI is a heterogeneous group. The bacilli of this group have in common moderate resistance to isoniazid, catalase negativity, and good growth in 10 γ of streptomycin and PAS. There was considerable variability in growth in 100 γ of streptomycin and PAS, so that three subgroups may be made. At the time these infections occurred, 5 patients with drug-resistant bacilli were present. From figure 2, *RU* appears to have bacilli similar

TABLE 5

RESISTANCE RESULTS IN GROUPS V, VI, VII

Animal Number or Patient	Culture Date	Catalase	Streptomycin		Isoniazid			PAS	
			10 γ	100 γ	1.0 γ	5.0 γ	25 γ	10 γ	100 γ
Group V									
40	3/6/58	+	N	N	15c*	N		3+	2+
DA	12/14/57	+	N	N	1+	N		3+	1+
	1/13/57	+	N	N	3+	1+		3+	1+
MA	12/9/57	+	N	N	N	N		1+	N
	4/14/58	+	N	N	4+	1+		4+	3+
Group VI									
A.48	5/27/58	N	4+	4+	2+	25c	N	2+	N
49	5/27/58	N	4+	2+	2+	1+	N	2+	N
B.50	5/27/58	N	4+	N	3+	1+	N	2+	N
C.51	6/29/58	N	3+	N	4+	1+	3c	3+	2+
52	6/29/58	N	3+	N	4+	1+	N	3+	2+
53	6/29/58	N	4+	N	4+	20c	N	4+	2+
54	6/29/58	N	3+	N	3+	1+	10c	3+	2+
55	7/14/58	N	4+		3+	2+		4+	
RU	10/16/57	N	4+	3+	4+	2+	1+	2+	N
	1/13/58	N	4+	2+	1+	1+	10c	1+	N
	5/5/58	N	4+	4+	4+	1+	N	3+	10c
Group VII									
57	7/25/58	+	N		N	N		N	

* c = colonies
Catalase negative indicated by N
Catalase positive indicated by +

to those in *Group VI*. However, detailed presentation in tables 5 and 6 shows that agreement is poor. *RU*'s bacilli showed drug resistance similar only to that of infections Nos. 48 and 49.

If patient *RU* was the source of these two infections, it may be wondered why during his long residence only 2 animals were infected. In this regard it may be pertinent that in April and May, 1958, after many months of fair control with pyrazinamide, this patient's condition showed definite worsening. In mid-May, kanamycin therapy was instituted, with definite improvement. Indeed, on July 14 and July 29 negative smears and one negative culture were obtained. Improvement was maintained until November, 1958. Aside from this possible explanation for infections Nos. 48 and 49, the source of these infections of *Group VI* remains obscure.

Group VII is infection No. 57, from which drug-susceptible bacilli were isolated; it probably came from one of the several patients with drug-susceptible bacilli, as shown in figure 2.

Group VIII is presented in table 6. Although figure 2 makes this group appear similar to that of *Groups I* and *VI*, inspection of the susceptibility details in table 6 will show that this is a distinct group. Culture of this material was done, undiluted and diluted 10^{-1}, 10^{-2}, 10^{-3}. In all dilutions growth in streptomycin and isoniazid was equal to that in the similarly diluted controls.

For PAS in both one and 10 γ dilution, 10^{-1} eliminated all growth. This suggests that the variability of growth in 10 γ of PAS is a reflection of sampling of a heterogeneously PAS-resistant group of bacilli, but that in all the infections, Nos. 48 through 74, the resistant proportion of the population was essentially constant. The results of resistance studies done on the sputum of the 6 patients with resistant bacilli who were present at the time when these infections of *Group VIII* occurred are also set forth in table 6.

That patient *GA* was the source of these infections is indicated by several factors. Not only is the pattern of drug resistance of his bacilli similar to that of the bacilli isolated from the animals, but on dilution his bacilli showed amounts of growth similar to the bacilli from the animal infections, i.e., the portion of his bacterial population resistant to PAS was the same as that of the bacilli isolated from the guinea pigs; none

TABLE 6
RESISTANCE RESULTS IN GROUP VIII

Animal Number or Patient	Culture Date	Catalase	Streptomycin		Isoniazid				PAS	
			3.5 γ	10 γ	0.2 γ	1.0 γ	5 γ	10 γ	1 γ	10 γ
58	9/2/58	N	4+	4+	4+	27c*	48c	35c	4+	15c
61	9/26/58	N	4+	4+	4+	1+	39c	43c	4+	N
62	9/26/58	N	4+	4+	4+	39c	34c	1+	4+	2+
63	9/26/58	N	4+	4+	4+	16c	20c	6c	4+	N
64	9/26/58	N	4+	4+	4+	4+	1+	1+	4+	2+
65	9/26/58	N	4+	4+	4+	44c	25c	22c	3+	1+
66	10/2/58	N	4+	4+	4+	1+	1+	1+	3+	N
67	10/2/58	N	4+	4+	4+	1+	21c	17c	2+	1+
68	10/2/58	N	4+	4+	4+	1+	1+	1+	2+	N
69	10/2/58	N	4+	4+	4+	1+	1+	1+	3+	2+
70	10/2/58	N	4+	4+	4+	45c	47c	12c	4+	1+
71	10/17/58	N	4+	4+	4+	3+	N	1c	3+	
72	10/17/58	N	4+	2+	4+	1+	1+	30c	3+	1+
73	10/17/58	N	4+	4+	3+	11c	10c	9c	3+	N
74	10/17/58	N	4+	4+	4+	14c	19c	4c	3+	N
76	11/10/50	N	4+	4+	4+	2+	2+	2+	4+	4+
RU	7/30/58	N	4+	4+	4+	3+	2+	1+	3+	1+
GA	8/27/58	N	4+	4+	4+	3+	2+	1+	3+	1+
	12/15/58	N	4+	4+†	4+	4+	10c	‡	§	5c
FR	9/24/58	N	4+	4+	4+	4+	4+			4+
ST	9/22/58	+	3+	2+	4+	4+	4+		4+	3+
BU	9/22/58	N	4+	4+	4+	4+	4+		3+	1+
WA	9/22/58	N	4+	3+	4+	4+	4+	4+	4+	4+

* c = colonies
† 4+ in 100 γ of streptomycin
‡ Negative in 25 γ of isoniazid
§ 2+ in 5 γ of PAS
Catalase negative indicated by N
Catalase positive indicated by +

TABLE 7
RESISTANCE RESULTS IN GROUP IX

Animal Number or Patient	Culture Date	Catalase	Streptomycin		Isoniazid				PAS	
			3.5 γ	10 γ	0.2 γ	1.0 γ	5 γ	10 γ	1 γ	10 γ
77	12/12/58	+	4+	N	7c*	N	N	N	4+	4+
RE	3/18/59	+	2+	N	N	N	N		2+	1+

*c = colonies
Catalase negative indicated by N
Catalase positive indicated by +

of the other 6 patients had bacilli which showed such a growth pattern. The time relationship between the presence of *GA* and the onset of the infections is remarkably good. The first animal infection was found four weeks after his admission, and the last of the series of sixteen infections occurred during his last week of residence. In contrast, *WI* and *RU* had been in residence many months; *FR*, *BU*, *WA*, and *ST* entered the ward after the series of infections started, and left either before or after it ended.

Group IX is infection No. 77, which probably occurred in October, 1958 (table 7). The animal infection was produced with a catalase-positive organism completely susceptible to isoniazid, but showing 4-plus growth in 10 γ of PAS and 4-plus

growth in 3.5 γ of streptomycin. There was no growth in 10 γ of streptomycin. Patient *RE* was the only patient with such bacilli present in the ward at the time of this infection. Patient *BA*'s organisms showed 4-plus growth in 100 γ of streptomycin, and his time of residence is too early to have caused this infection.

Discussion

A striking feature of this study is the conclusion that 35 of 48 cultured air-borne infections came from 3 patients, even though 77 patients occupied the ward during the period under consideration. This conclusion will be discussed from two points of view: first, how secure is the evidence on which it is based; and second, what are some of the chief factors which may account for it. Among these factors, an explanation will be sought for the remarkable predominance of drug-resistant organisms in the infected guinea pigs.

After this study had been in progress for a few months, it was noted that infections occurred shortly after a particular patient (*AR*) entered the ward, continued during his prolonged residence on the ward, and terminated when he left the ward. On the basis of this obvious temporal association, it was suspected that this patient might be exceptionally infectious before the possibility of identifying the source of the infections by the drug-resistance characteristics of the organisms was seriously considered. In this same way the other 2 highly infectious patients (*JO* and *GA*) were also suspected. Subsequently, the matching of the catalase-activity and drug-resistance characteristics of the organisms in the guinea pig with the organisms in the patients' sputum supported the earlier suspicions. The writers have thus come to have considerable confidence in the matching technique under the conditions of the present experiment.

The fact that patient sources could not be identified in 6 of 48 animal infections is not surprising when it is realized that the patients' bacilli were tested for drug resistance only at three-month intervals. During the intervening period, bacilli with drug-resistance characteristics different from those exhibited by the routine cultures might well have been coughed into the air (10). This explanation seems very much more plausible than the alternative possibility that these organisms arose from casual visitors to the ward. It seems unlikely that such visitors would be producing large numbers of drug-resistant organisms and, even if they were, the duration of visits by any individual did not exceed 2 per cent of the total time of animal exposure. In this brief period, even the most infectious patients would probably never have infected a guinea pig.

Riley and associates (2) have considered in detail the possibilities that animal infections might have arisen from unsuspected sources outside of the pilot ward and that cross infections between animals might have occurred. They present impressive evidence against such possibilities. Since their paper was published, further evidence that ward air was in fact the source of the animal infections has been obtained by heavily irradiating the air going from the ward to the animal exposure chamber with ultraviolet light. With the air sterilized in this way, animal infections have ceased completely. The likelihood of cross infection has been further evaluated by deliberately putting a few animals previously infected with air-borne tuberculosis into the exposure chamber receiving irradiated air. The infected animals were removed one month later when their tuberculin tests became positive. They thus occupied the exposure chamber during the initial stages of their disease exactly as did the animals who were infected by breathing unirradiated ward air. No cross infections occurred. It thus appears reasonably sure that the guinea pig infections truly reflect both the numbers and the strains of organisms transmitted by the air from the patients on the ward.

The question of infectiousness is far too complicated to explain on the basis of the present data. There are, however, a number of factors which can be mentioned, and some of these seem very likely to have been important in shaping the results of the present study. These factors are divided into two groups: those related to the number of organisms put into air-borne suspension by the patient, and those related to the viability of the organisms in the air-borne state.

The number of organisms put into air-borne suspension by the patient depends in part upon the effectiveness of the patient as an atomizer and in part upon the character of the material atomized. Effectiveness as an atomizer has to do with the amount of coughing, the violence of the cough, and the care with which the mouth is covered while coughing. The character of the material atomized has to do with the concen-

tration of organisms in the sputum, the amount of sputum, and the physical characteristics of the sputum.

In this study a truly quantitative evaluation of these factors was not made. After August, 1958, all of the study patients were cared for by one physician and an attempt was made to gain a clinical estimate of the infectivity of each patient, based on cough and quantity and quality of sputum. It was thought that *GA* almost certainly would be a highly infectious patient. *JO*'s cough and sputum production were comparable with or exceeded by those of at least 11 other patients, including *BY*, *SM*, *HI*, and *RU*. However, his sputum was noted to be exceptionally liquid. It is known that *AR*, *JO*, and *GA* had the most consistently high Gaffky counts of any of the patients in the study.

From a review of the sputum studies as given in table 1, it is evident that patients harboring resistant bacilli provided 1.4 times more days of sputum positive on microscopy and 1.2 times more days of positive cultures than the patients with drug-susceptible infections. When the comparison is made between the days of highly positive smears and cultures, the figures are 1.6 and 2.4, respectively. In contrast to this, the animal infections with resistant bacilli were 23 times more frequent than infections with drug-susceptible bacilli. The magnitude of this difference suggests that factors other than differences in the number of bacilli were of importance.

The rigors of life in the air-borne state probably bring early death to the vast majority of tubercle bacilli which are coughed into the air. Ratcliffe (11) estimates that more than 99 per cent are lost almost immediately, leaving but a fraction of 1 per cent surviving for a matter of hours. Survival is known to be dependent to an important degree on the other constituents of the droplet in which the organism is atomized. Thus, when a suspension of organisms in culture medium is atomized, many more organisms survive than when a suspension in saline or water is atomized. It seems likely that the presence of antibacterial substances in the suspensory medium might also influence the survival of organisms after atomization.

All of the patients in the present study were being treated with antimycobacterial drugs, and the droplets which were aerosolized when the patients coughed presumably contained minute amounts of the drugs. Concentrations of isoniazid approximating those in the blood have been found in the saliva and sputum of patients under treatment (12, 13) and streptomycin concentrations as high as 7.7 γ per ml. have been found in cavity contents (14). Droplets containing some such concentrations of drug along with a tubercle bacillus must evaporate in a fraction of a second in order to remain air borne. In the process of evaporation, the volume of the droplet must shrink several thousand fold, and the concentration of drug must simultaneously increase several thousand fold. Consequently, in the air-borne droplet nuclei, the tubercle bacilli from the patients in the present study were presumably exposed, not only to the normal rigors associated with dehydration, but also to the presence of very high concentrations of antimycobacterial drugs. Whether the drug molecules could exert their characteristic antimicrobial action in these circumstances has not yet been subjected to study.

It is clear that, despite their greater pathogenicity for the guinea pig (15–18), most of the drug-susceptible organisms failed to transmit infection, and it is possible that most of them may have been killed as a result of their exposure to high concentrations of drug in the air-borne droplet nucleus. This would explain the preponderance of infections by drug-resistant organisms, but would not explain the variability of infectiousness among the patients harboring similar drug-resistant strains. Here, the explanation of the great infectiousness of a few patients might be found in three general areas: (*1*) major differences between patients in the number of organisms put into air-borne suspension; (*2*) a high ratio of drug resistance of the organism to the actual concentration of drug to which it was exposed; (*3*) ability of certain organisms to undergo adaptive changes favoring survival in air. In regard to the second point, it is of interest that all patients identified as sources of infection were receiving only drugs which they had received for many months and which failed to "convert" their sputum to negative or to alter their roentgenographic findings; however, they were not unique in this respect.

Coburn has noted that dissemination of streptococcal disease does not seem to be related to the number of cocci harbored by the carrier, but rather to adaptive changes in the organisms

which enable it to survive in the air-borne state and to multiply once implanted (19). A similar state of affairs might exist in the case of tuberculosis, and there may be unusually dangerous disseminators of tubercle bacilli just as there are dangerous disseminators of staphylococci and streptococci.

The reason drug resistance dominates the picture in the present study may well be because all of the patients were receiving chemotherapy. Preliminary studies in this laboratory show, as might be expected, that untreated patients with drug-susceptible organisms can be dangerous disseminators. As yet there is no information on the variability of infectiousness among untreated patients. This question is of great epidemiologic importance since, for the most part, tuberculosis is spread in the community by patients with unsuspected and untreated disease whose organisms are not resistant to antituberculous drugs.

Summary

During a two-year study of air-borne tuberculosis, infection was transmitted to 71 animals. Seventy-seven patients participated in the study. Of the 48 animal infections which were cultured and in which drug-resistance studies were done, it was found that 35 of the infections had been caused by 3 patients. Possible reasons for the remarkable infectivity of these 3 patients are discussed. It is suggested that this infectivity was related to the patients' ability to produce large numbers of bacilli unusually capable of withstanding the rigors of air-borne transmission.

Sumario

Diseminadores de la Tuberculosis: Estudio de la Variabilidad en Infectividad Aérea de los Tuberculosos

Durante un estudio bienal de la tuberculosis transportada por el aire, se transmitió la infección a 71 animales. Setenta y siete enfermos participaron en el estudio. De las 48 infecciones en animales en que se hicieron cultivos y se realizaron estudios de la fármacorresistencia, se descubrió que 35 habían sido ocasionadas por 3 enfermos. Se exponen posibles razones de la notable infectividad de estos 3 enfermos. Señálase que esta infectividad se relacionó con la capacidad de los enfermos para producir grandes cantidades de bacilos, capaces éstos a su vez de tolerar los rigores de la transmisión por el aire.

Resume

Agents disséminant la tuberculose: une étude de la variabilité de l'infection par air des malades tuberculeux

Pendant une étude de deux ans de la tuberculose transmise par air, l'infection fut transmise à 71 animaux. Soixante dix sept malades participèrent à cette étude. Parmi les 48 infections animales dont on fit la culture et chez qui on étudia la résistance aux médicaments, on trouva que 35 de ces infections avaient été causées par 3 malades. Les auteurs discutent les raisons possibles de la contagiosité remarquable de ces 3 malades. Ils ont suggéré que cette contagiosité était en relation avec la posibilité de ces malades de produire de grandes quantités de bacilles capables de supporter les rigueurs de la transmission par air.

Acknowledgment

The technical assistance of Mr. N. Weinstock and Mrs. F. Wittstadt is gratefully acknowledged.

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THE BACTERIOLOGY OF RESECTED TUBERCULOUS PULMONARY LESIONS[1,2]

II. Observations on Bacilli Which are Stainable but Which Cannot be Cultured

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Introduction

The observation of Medlar and associates (1) that some resected tuberculous lesions contain readily stainable bacilli which cannot be cultured has been amply confirmed by many investigators (2–4). In a previous paper, the occurrence of this phenomenon was reported in 16 per cent of forty-five resected tuberculous lung specimens from patients with open cavities, and in 58 per cent of one hundred and ten resected specimens from patients with closed lesions only (2). It has not been conclusively proved that these nonculturable bacilli are dead. In this situation it is only possible to prove life; failure to "revive" these organisms is not proof that they are dead, but may merely indicate that inadequate viability tests were employed. From a practical point of view, the greater the number of different concepts and techniques that are employed unsuccessfully for attempting to revive these organisms, the greater is the likelihood that they are dead.

The present report is concerned with some of the results of a six-year study of the state of viability of stainable tubercle bacilli in lesions resected from human lungs. A number of special techniques were tested for their ability to revive bacilli which could be seen, but could not be cultured by standard methods. The goal of this project was not a slight increase in the number of positive cultures but, rather, the hope of a dramatic recovery of viable organisms from a large proportion of the specimens positive on microscopy which were negative on culture by standard methods. None of the techniques tested succeeded in reviving the bacilli.

Another approach to this problem has been an investigation of the persistence of acid-fast bacilli in caseum incubated under anaerobic conditions. The rate of disappearance of native acid-fast bacilli from caseum has been estimated in an effort to determine whether autolysis is the predominant mode of elimination of bacilli from lesions.

Methods and Materials

Preparation of Specimens

Resected tissue was homogenized and inoculated on medium on the day of surgery, unless otherwise indicated. The selection of material and homogenization methods have been described previously (2). Because of limited amounts of pathologic material available for such a study, no single special technique was used for the whole series.

Revival Attempts

Control cultures: In all tables of results, the yields obtained by various test techniques are compared with control cultures, which were always made in parallel with the test. Two cultures represented the controls: a 15-ml. prescription bottle slant of Löwenstein-Jensen medium and another of Petragnani medium. The controls each received 0.1 ml. of the tissue homogenate.

Charcoal suspensions: On the chance that an adsorbable inhibitory material was present in the tissue homogenate, a series of homogenates was mixed with equal volumes of sterile 1 per cent aqueous suspensions of activated charcoal.[3] After standing one hour, the charcoal-treated specimens were inoculated directly onto Löwenstein-Jensen medium.

Modified Hobby technique: Hobby and associates (5, 6) have reported success in reviving tubercle bacilli by freezing large pieces of tissue, thawing and grinding the tissue, washing the homogenates in broth, and long-term incubation of these large inocula and washings in liquid medium. A series of specimens has been similarly treated in the present study for evaluation of the technique. It must be pointed out, however, that Dr. Hobby's method has not been followed exactly (e.g., smaller amounts of tissue were used, and penicillin was omitted from the wash fluids); thus, only certain aspects of her special treatment were tested. The specimens received here were homogenized imme-

[1] From the Microbiological Research Unit, Veterans Administration Hospital, San Fernando, California.

[2] Presented in part before the Medical Session, as part of *Section 2B*, at the annual meeting of the American Trudeau Society, Chicago, Illinois, May 25, 1959.

[3] Merck, U.S.P.

diately on receipt from surgery, and small aliquots (about 0.2 ml.) were then frozen. When a suitable number of specimens thus treated were accumulated, the preparations were thawed, centrifuged, and washed three times in Dubos broth without Tween® 80. Penicillin was not used. The three washings were pooled and incubated. The sediments were resuspended and distributed equally to one tube each of Dubos broth with and without Tween 80 and to a Löwenstein-Jensen slant. All tubes were incubated for nine months, with monthly sampling of the liquid cultures to Löwenstein-Jensen slants for the first three months, and again at six and nine months.

Broth culture: For this study, 0.1 ml. of homogenate was inoculated directly to screw-capped test tubes containing 6 ml. of Dubos Tween 80–albumin broth, and incubated at 37°C. Subcultures were made to Löwenstein-Jensen slants to confirm growth.

Biphasic culture: Bernstein and associates (7) have reported the use of a biphasic culture, employing an agar medium as the solid phase and Dubos broth as the liquid. In this study, Löwenstein-Jensen medium was used as the solid phase (4 ml. slanted in a 15-ml. prescription bottle) in order to take advantage of the detoxifying effects of the egg phospholipids. Four milliliters of Dubos Tween 80–albumin broth comprised the liquid phase. The bottles received 0.1 ml. of tissue homogenate, and were incubated upright at 37°C. Every three weeks the bottles were tipped to reinoculate the solid medium.

Phthiocol: Inasmuch as phthiocol has been reported to be a mycobacterial growth factor (8), the use of this compound was undertaken to determine its effect on the nonculturable tubercle bacilli being studied here.

A solution of 10 mg. per ml. of phthiocol in 95 per cent ethanol was diluted 1:200 in sterile albumin fraction V solution (0.5 per cent). One-tenth of a milliliter of this solution was mixed with 0.1 ml. of tissue homogenate, and this mixture was plated to Löwenstein-Jensen slants. Based on a 4–5-ml. volume of the egg medium per slant, the phthiocol, on diffusion, yielded a final concentration of about 1γ per ml.

Pyruvate: Dubos (9) has demonstrated that pyruvate antagonizes the lethal effects of anaerobiosis and high carbon dioxide tensions on tubercle bacilli *in vitro*. An attempt was made, therefore, to revive bacilli in resected lesions by pyruvate treatment. Sodium pyruvate was suspended to a concentration of 0.025 M in 95 per cent ethanol containing 0.02 per cent Tween 80. Dilute hydrochloric acid was used to adjust the pH to 4.0, at which point the pyruvic acid went into solution. Aliquots containing 0.1 ml. of the sterile pyruvic acid solution were distributed to micro tubes, evaporated to dryness *in vacuo*, and stored in the freezer. Aliquots containing 0.1 ml. of the tissue homogenates to be tested were added to these samples, and incubated for one hour. The preparations were then inoculated onto Löwenstein-Jensen slants.

Modifications of gaseous environment: A brief summary of this phase of the study has been published (10). The homogenates were inoculated onto Löwenstein-Jensen slants, and the screw caps were replaced with cotton plugs. A 2-inch length of sterile rubber tubing was placed over the neck of each culture bottle and the other end was sealed with a vaccine stopper. A needle was introduced through the stopper, sufficient air was removed, and gas was admitted, to yield the following gaseous mixtures in the culture bottles:

(*a*) Air diluted with oxygen to yield a final oxygen concentration of 50 per cent;
(*b*) Air diluted with nitrogen to yield a final oxygen concentration of 5 per cent;
(*c*) Air diluted with carbon dioxide to yield a final oxygen concentration of 16 per cent, and a carbon dioxide concentration of 20 per cent.

Reinoculation of washings from negative slants: On the chance that early growth may have occurred and then aborted before development of visible colonies, a series of apparently negative Löwenstein-Jensen and Petragnani slants were washed and scraped in the presence of sterile water, and the washings were replated onto fresh Löwenstein-Jensen slants.

On the same principle as stated above, another set of negative slants was flooded with 5 ml. of Dubos Tween-albumin broth which was incubated for two weeks in contact with the original egg medium. Subcultures were then made onto fresh Löwenstein-Jensen slants.

Prolonged incubation: It has been the practice in this laboratory to examine cultures weekly for a full twelve weeks of incubation. Cultures which were negative at twelve weeks were saved and reexamined at irregular intervals up to two years.

Guinea pig inoculations: Tissue homogenates were inoculated into guinea pigs by various routes and with various special treatments. The following guinea pig techniques were studied:

(*a*) Inoculation by the subcutaneous route, in the groin
(*b*) Inoculation by the intratesticular route
(*c*) Inoculation by the intracardiac route
(*d*) Inoculation by the subcutaneous route, followed by daily intramuscular injection of 2.5 mg. of cortisone[4]
(*e*) Subcutaneous inoculation of 0.1 ml. of tissue homogenate mixed with 1 ml. of a 5 per cent aqueous suspension of silica (3 μ particles or smaller[5]).

Animals surviving eight weeks after inoculations were skin-tested with 0.1 ml. of 1:20 Old Tuberculin, sacrificed, and autopsied.

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[4] Merck, Cortone® Acetate.

[5] Obtained through the courtesy of Dr. G. W. H. Schepers, of the Saranac Laboratory, Saranac Lake, New York.

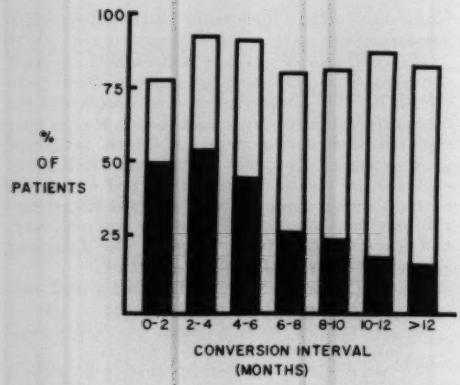

FIG. 1. Recovery of visible and viable tubercle bacilli from resected closed lesions. Patients' sputum had been negative for varying periods of time, prior to surgery; the period of presurgical negativity is designated "conversion interval." The total height of each bar represents the proportion of patients, in a given conversion interval group, whose lesions yielded visible tubercle bacilli, regardless of viability; the solid portions of the bars represent the proportion of patients from whom viable organisms were recovered.

Anaerobic Incubation of Tissue Homogenates

A study was made of the persistence of acid-fast bacilli in homogenized tuberculous tissue after prolonged incubation under anaerobic conditions. The purpose of this study was to determine whether these acid-fast bodies might be expected to disappear from closed lesions by some autolytic mechanism, without continuous exposure to dynamic forces of the host.

Aliquots containing 0.1 ml. of homogenized resected lesions were distributed to 7 × 50 mm. sterile test tubes, overlaid with sterile mineral oil, cotton-stoppered, and incubated at 37°C. for periods up to two years. Smears were made at various times from these homogenates, and counts of acid-fast bacilli were compared to the counts of bacilli present in the original fresh homogenates.

The homogenates were smeared over a 10^2-cm. area of a slide, air dried, and stained for acid-fast bacilli. Five hundred fields were examined per slide; by this technique, a "negative" specimen may be more accurately stated to contain less than one bacillus in 500 fields, or 80 bacilli per smear. In order to minimize counting errors introduced by specimens with very few bacilli present initially, however, the analysis is presented only for specimens bearing at least 4,000 bacilli on the smear from the fresh homogenate. This is represented by a count of at least one bacillus per ten fields examined.

In Vitro Autolytic Systems

The problem of persistence of nonculturable acid-fast bacilli in lesions places fresh emphasis on the study of autolytic mechanisms in tubercle bacilli. The results of a few experiments are presented here to demonstrate the effect of age of a culture on autolysis under adverse conditions.

Cultures of *M. tuberculosis* H37Rv were grown in Dubos Tween-albumin medium. In one set of experiments, triplicate cultures were sealed with rubber vaccine stoppers after ten, twenty, and forty days of incubation. Needles were introduced through the stoppers and the tubes were flushed with carbon dioxide. In another set of experiments, streptomycin was added to a concentration of 20 γ per ml. to replicate sets of cultures after ten, twenty, and forty days of incubation. All cultures were then reincubated. Growth and lytic curves were obtained by plotting optical densities obtained on a Lumetron colorimeter at 580 mμ.

RESULTS

Analysis of the Problem

In a prior report (2) an inverse relationship was demonstrated between the bacterial population of a resected specimen and the interval between the last positive sputum or gastric lavage specimen obtained from a patient, and subsequent resection. This interval was designated the "conversion interval," and was expressed in months. Statistical analyses have been made of the relationship between the conversion interval and the nature of the bacterial population in resected lung tissue from 202 patients who had *closed* lesions only. This relationship is illustrated in figure 1. There is no significant decline in the proportion of patients yielding visible bacilli, regardless of length of conversion interval (correlation coefficient of the conversion interval against the proportion of patients: $r = -0.17$). On the other hand, the proportion of patients whose visible bacilli are culturable is inversely related to the conversion interval ($r = -0.83$ for 164 patients with conversion intervals ranging from zero to twelve months; for the remaining 38 patients, with conversion intervals ranging from thirteen to forty-six months, $r = 0.05$). It appears, then, that for the first twelve months after sputum conversion, the bacilli steadily lose their ability to reproduce, but they do not tend to disappear.

Further analysis was made of the *actual numbers* of acid-fast bacilli seen in resection homogenate smears from patients whose bacilli could not be grown in culture. Again, only closed-

TABLE 1

COMPARISON OF CONTROL CULTURES AND SPECIAL ATTEMPTED REVIVAL TECHNIQUES

Special Treatment	Total Number of Specimens	Number of Positive Cultures on:				Total Number of Positive Specimens by Culture or Guinea Pig Inoculation
		Control Culture only	Special Treatment only	Both	Neither	
Charcoal suspensions	40	2	3	10	25	15
Modified Hobby technique	43	0	0	13	30	13
Broth culture	52	2	0	19	31	21
Biphasic culture	102	6	1	29	66	36
Phthiocol	10	0	0	4	6	4
Pyruvate	25	2	1	11	11	14
50 per cent oxygen	29	3	5	9	12	17
5 per cent oxygen	29	7	1	8	13	16
20 per cent carbon dioxide	30	8	0	8	14	16
Guinea pig, subcutaneous inoculation	58	5	1	21	31	27
Guinea pig, intratesticular inoculation	49	4	1	20	24	25
Guinea pig, intracardiac inoculation	40	1	0	18	21	19
Guinea pig, cortisone treated	23	1	1	6	15	8
Guinea pig, silica treated	30	1	0	15	14	16

Only specimens containing stainable acid-fast bacilli are included in this table.

lesion specimens are reported. The method of counting bacilli was reported previously (2); the numbers of bacilli are expressed as logarithm of count in 0.1 ml. of tissue homogenate. The mean logarithmic value in 107 specimens that were positive on microscopy and negative on culture was 3.7, i.e., the geometric mean count was about 50,000 bacilli per ml. of homogenate. Statistical analysis indicated no significant correlation between conversion interval and count of visible bacilli ($r = 0.08$). This further corroborated the conclusion that tubercle bacilli usually do not fade away, as long as the lesion itself persists, even though bacilli may no longer be cultured.

Revival Attempts

Since this study was concerned with the visible but not culturable bacilli, the results are reported only for those specimens in which acid-fast bacilli could be seen on microscopy of the homogenates. The results of various techniques are recorded in table 1.

The results of prolonged incubation and of washing and replating of negative cultures are not tabulated. Ninety-six apparently negative slants, which had been incubating for three to ten months, were washed and scraped in the presence of distilled water, and the washings were replanted onto fresh medium. After twelve weeks of incubation of the fresh slants, none had any growth. One hundred and forty apparently negative slants, which had been incubated for three to fourteen months, were reincubated for two weeks with 5 ml. of Dubos Tween-albumin broth added, and aliquots of the broth were then plated to fresh Löwenstein-Jensen slants. After ten weeks of incubation of the fresh slants, none had any growth.

The following observations have been made of prolonged incubation of the original cultures. Of 138 negative slants followed for nine months beyond the normal three-month observation period, none became positive during the extended study period. None of 54 negative slants followed for six to nine months beyond the normal three-month observation period became positive during the extended study period. Of 46 negative slants followed for three to six months beyond the normal three-month observation period, none became positive during the extended study period. Four of 36 negative slants followed for less than three months beyond the normal three-month observation period became positive during the extended study period. Two cultures from one specimen each required thirteen weeks for growth. One slant had one colony and the other had 3. One slant from another specimen exhibited one colony

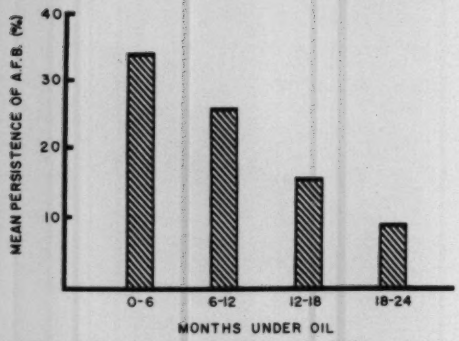

FIG. 2. Persistence of acid-fast bacilli in original tissue homogenates after varying periods of incubation under mineral oil. Persistence is expressed as the mean percentage of number of acid-fast bacilli still visible when compared with the acid-fast bacilli count in the original fresh homogenate.

at fourteen weeks. All of the other slants remained negative. A Löwenstein-Jensen slant from still another specimen yielded 3 colonies sometime between the fourth and sixth months post inoculation. The Petragnani control from the same specimen had shown ample growth at two weeks. The delayed growth here was probably due to a faulty bottle of medium.

Among the 360 sets of paired (control and special treatment) cultures recorded in table 1, 152 yielded growth in one or both tubes. Discrepancies occurred between the two tubes of a set in 41 sets. Of the 11 sets in which only the special culture was positive, 9 had 10 or fewer colonies, and the other 2 had 15 and 20 colonies, respectively; the discrepancy can be explained by chance distribution of the few viable bacilli in the inocula. Similarly, of 30 sets in which only the control culture was positive, 23 tubes had fewer than 10 colonies, 4 had between 10 and 30 colonies, and only 3 had more than 50 colonies. Again, the discrepancies may be largely explained by chance distribution of a few bacilli.

Among the 200 paired control culture and guinea pig sets, 95 yielded viable organisms. Discrepancies occurred between the control culture and the guinea pig in 15 sets. In only 3 sets was the guinea pig positive and the control culture negative. Of the 12 sets in which only the culture was positive, 5 cultures had 3 or fewer colonies, and one had 32 colonies. Of the remaining 6 sets, with cultures carrying from 200 to more than 1,000 colonies, the cultures were all resistant to more than 5 γ of isoniazid per ml., which would explain the lack of pathogenicity for the guinea pig.

None of the special treatments was demonstrated to revive nonculturable tubercle bacilli in resected lesions.

Anaerobic Incubation of Tissue Homogenates

The tissue homogenates were incubated under oil for periods up to two years. Analyses were made of acid-fast counts from a total of 62 smears prepared from homogenates which, before incubation under oil, had 4,000 or more acid-fast bacilli per slide.

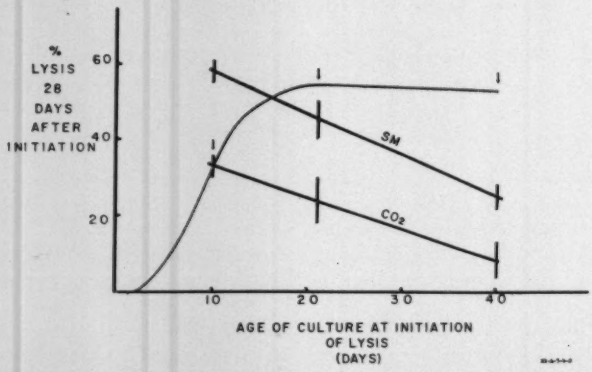

FIG. 3. Extent of autolysis of tubercle bacilli after exposure to streptomycin or flushing with carbon dioxide at various points on the growth curve. The solid lines represent percentage of lysis, as calculated from reduction of optical density. The dotted line represents the normal growth curve, and is included to supply reference points for the various treatments.

The proportion of specimens in which acid-fast bacilli could still be demonstrated, regardless of actual numbers of bacilli seen, ranged from 89 to 96 per cent; among 17 specimens incubated under oil for 18 to 24 months, bacilli could still be seen in 16.

A further analysis was made for correlation between the time under oil and the persistence of bacilli, expressed in percentage of *actual numbers* of bacilli originally present in each fresh homogenate. A negative correlation was found to exist ($r = -0.40$). Thus, during the first six months under oil, a mean decrease of 66 per cent in bacillary count occurred. During each of three subsequent six-month periods under oil, approximately 8 to 10 per cent of further decreases occurred in a linear manner (figure 2).

In Vitro Autolytic Systems

In figure 3, the effect of age on the autolytic capacity of tubercle bacilli in a carbon dioxide environment and of bacilli under the influence of streptomycin are illustrated. Lysis is most marked in cultures in the most active growth phase, it is reduced as growth terminates, and it is minimal in cultures which have terminated multiplication nineteen days prior to treatment with carbon dioxide or with streptomycin.

Discussion

In a recent paper, McDermott (11) distinguished between "dormant" and "latent" infection. Dormancy implied the demonstrable presence of viable pathogens, without active disease; latency, on the other hand, implied the presence of pathogens which could not be demonstrated by microscopic examination or by culture. In either case, some change in the host's susceptibility could give rise to active disease caused by the endogenous latent or dormant pathogens. In the case of the closed tuberculous lesion in man, we may be dealing with an intermediate situation. The term "latency" is not applicable, since the bacilli can be demonstrated on microscopy. The term "dormancy" would be applicable only if it could be demonstrated that these visible bacilli were viable, even though they cannot be grown by standard culture techniques.

A number of *in vitro* and *in vivo* attempts to revive these organisms have been described in the present study. None was successful. Incubation of resected tissue homogenates under oil demonstrated that the acid-fast bacilli can persist and be recognized for at least two years. Samplings from such homogenates known to have harbored culturable bacilli when first prepared indicated, however, that the bacilli lost their culturability, in most cases, in two months or less. These findings are compatible with Steenken's observations (12) that heat-killed H37Rv tubercle bacilli persisted for long periods after inoculation into guinea pigs, and that live, avirulent H37Ra bacilli were stainable long after most of the inoculated bacilli could no longer be cultured.

The turbidity experiments on liquid cultures showed that the autolysis of tubercle bacilli subjected to adverse conditions is dependent to some extent on the physiologic age of the cultures.

Thus it may be seen that, under appropriate conditions, bacilli may die and disappear; yet, under other conditions, they may lose the ability to reproduce in culture but retain their structural and tinctorial integrity. It cannot be proved, however, that they are dead. Further evidence on the viability potential of such organisms may be provided by evaluation of various enzymatic activities of these bacilli by cytochemical means. Such studies are presently being initiated. Long-term follow-up studies on unresected patients must be continued in order to provide information on reactivation of lesions.

The role of chemotherapy in producing the positive-on-microscopy but negative-on-culture phenomenon is difficult to assess. A series of 63 resections was examined which met the following criteria: (*a*) at least two distinct lesions existed in the resected tissue, (*b*) acid-fast bacilli could be seen microscopically in at least two of the lesions from each specimen, and (*c*) tubercle bacilli could be cultured from at least one lesion in each specimen. In 36 of these specimens, all of the lesions yielded positive cultures; in the remaining 27 specimens, the positive culture lesion was associated with other lesions which yielded specimens positive on microscopy but negative on culture. With this background information, it may be seen how difficult it would be to attribute the positive-on-microscopy but negative-on-culture phenomenon to a particular drug regimen, unless one could ascertain the particular stage each lesion was in at the time of introduction of chemotherapy.

There is still a diversity of opinion about the

role of surgical resection of "residual" lesions. The hazards of surgery and the loss of pulmonary reserve must be weighed against the likelihood of reactivation of closed lesions. The presence and state of viability of tubercle bacilli in these lesions is a key factor in this problem.

In patients with closed lesions and no open cavities, the conversion of sputum generally corresponds to initiation of a process of decline in culturable bacilli within the lesion (*see* figure 1 and reference 2). The reduction in the proportion of patients with culturable organisms continues for a period up to twelve months after reversal of infectiousness of the pulmonary secretions. The situation then stabilizes, and a fairly constant proportion of patients (about 20 per cent) coming to surgery ten months or more after apparent sputum conversion yield viable organisms from their resected lesions. The term "apparent sputum conversion" is used advisedly. In a comparative study of digestion techniques (to be published at a later date), it has been noted that sputum or gastric lavage specimens with low Gaffky counts on microscopy yielded far fewer positive cultures than did those with higher Gaffky counts after treatment by standard digestion techniques. However, after treatment of sputum by a modified Patterson TSP-Zephiran technique (13), specimens in the Gaffky I and II ranges gave as many positive cultures as did those in the Gaffky IV and higher ranges. This suggests that some of the patients who were believed to have had noninfectious sputum for many months prior to surgery may actually have been excreting small numbers of bacilli during that time; the standard digestion techniques are too harsh to permit consistent recovery of very small numbers of bacilli in the secretions. From this it may be predicted that the proportion of patients with truly negative sputum (on microscopy or culture) for more than ten months preoperatively, who subsequently yield culturable bacilli from the resected tissue, may fall well below the 20 per cent indicated in figure 1.

In view of the current high degree of skill attained in pulmonary surgery, the hazards might be overshadowed by the advantages to be gained by removing closed lesions, 20 per cent of which harbor fully viable bacilli. If, however, improved sputum digestion methods remove a number of patients from the sputum-negative class, we may find that considerably less than 20 per cent of the "converted" patients yield culturable bacilli from their resected lesions. At some point the risk of leaving significant foci of viable bacilli in the lung may be balanced by the risks of surgery. At this point, a more subtle problem intrudes. How much importance should be attached to the finding of visible bacilli, which cannot be grown, in the resected tissue? Referring again to figure 1, it may be seen that the proportion of patients yielding visible bacilli on smear is not related to the conversion interval. Thus, 84 per cent of the patients who had resections more than twelve months after "apparent sputum conversion" show positive specimens on microscopy from the resected tissue. This proportion is not significantly different from that of patients who had positive specimens right up to the time of surgery. If these bacilli are dead, then they may be disregarded. If they are alive, however, i.e., potentially revivable, then there is indication for resection of such lesions in every case in which it is medically feasible. The question of whether the patients who had not had resection would represent a potential source of communicability would also need to be re-evaluated.

Summary

In a significant proportion of resected tuberculous lesions it is possible to demonstrate tubercle bacilli on microscopy, but the bacilli cannot be cultured.

A number of special *in vitro* and *in vivo* techniques have been employed in an attempt to revive these organisms. None has succeeded. However, tubercle bacilli could still be seen in tissue homogenates on microscopic examination after two years or more of anaerobic incubation.

The degree of autolysis of tubercle bacilli under the influence of streptomycin or carbon dioxide saturation in culture was dependent on the physiologic age of the cultures. Acid-fast bacilli can persist long after they have lost the ability to be grown by standard culture techniques. It cannot yet be proved that these bacilli are dead.

Sumario

La Bacteriología de las Lesiones Pulmonares Tuberculosas Resecadas. II. Observaciones sobre los Bacilos que son Colorables, pero No Cultivables

En una importante proporción de las lesiones tuberculosas resecadas, es posible descubrir bacilos tuberculosos en el frote, pero no cultivarlos.

Se han empleado varias técnicas especiales *in vitro* e *in vivo* para revivir estos microbios, pero ninguna ha dado resultado. No obstante, al cabo de dos años o más de incubación anaerobia todavía podían verse bacilos tuberculosos en los homogeneatos de tejido en frotes.

La intensidad de la autólisis de los bacilos tuberculosos bajo el influjo de la estreptomicina o de la saturación con bióxido de carbono en los cultivos dependía de la edad fisiológica de los cultivos. Los bacilos ácidorresistentes pueden persistir mucho tiempo de pués de haber perdido la capacidad para proliferar con las técnicas stándard de cultivo. No puede demostrarse todavía que estén muertos estos bacilos.

Resume

La bactériologie des lésions pulmonaires tuberculeuses réséquées. II. Observations de bacilles que l'on peut colorer mais pas cultiver

On peut démontrer, dans une proportion importante de lésions tuberculeuses réséquées, la présence de bacilles tuberculeux sur plaque, mais ces bacilles ne peuvant pas être cultivés.

Un nombre de techniques spéciales, *in vitro* et *in vivo*, a été employé pour tenter de faire revivre ces organismes. Aucune n'a eu de succès. Cependant, on put encore voir des bacilles tuberculeux sur les plaques de tissus homogénéisés, après deux ans ou plus d'incubation anaérobique.

Le degré d'autolyse des bacilles tuberculeux sous l'influence de la saturation en streptomycine ou en gaz carbonique dans la culture, dépendit de l'âge physiologique des cultures. Les bacilles acidorésistants peuvent persister longtemps après qu'ils ont perdu leur pouvoir de pousser avec les techniques ordinaires de culture. On ne peut pas encore prouver que ces bacilles sont morts.

Acknowledgment

The technical assistance of William J. Juarez and Eltha J. Nichols is gratefully acknowledged.

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OUTPATIENT CHEMOTHERAPY FOR TUBERCULOSIS[1]

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Introduction

Home treatment of tuberculosis with antituberculous chemotherapy has become increasingly popular both as a continuation of therapy following early hospital discharge and as an initial treatment of those who cannot or will not enter a sanatorium. The faithfulness of patients in following a prescribed chemotherapy regimen is then a matter of importance from the standpoint of: (*1*) emergence of drug-resistant organisms; (*2*) effectiveness of treatment; (*3*) public expense and wastefulness. The majority of outpatient treatment schedules are preceded by considerable public expense in case finding and/or hospitalization.

Mohler, Wallin, and Dreyfus (1) studied 245 patients with acute pharyngitis or otitis media due to beta hemolytic Streptococcus for whom outpatient treatment with oral penicillin for seven to ten days had been prescribed. On close questioning at the end of the course, 34.3 per cent admitted taking less than the prescribed amount. Of the 23 patients more than fifteen years of age, only 50 per cent took the medicine as prescribed.

Penman and Wraith (2) found that, of tuberculous outpatients supposed to be taking PAS, only 9 of 14 selected at random gave positive urine reactions for the drug.

Dixon, Stradling, and Wootton (3) found that, of 151 tuberculous outpatients supposedly taking PAS, only 76 (50 per cent) gave positive reactions when their urine was tested with Ehrlich's reagent. The duration of chemotherapy appeared to make litte difference in the percentage. They also found that judgments of the "health visitors" as to whether their patients were taking the drugs as prescribed were reliable in only one-third.

Pittman, Benzier, and Katz (4) reported that, of 61 outpatients studied with the Ehrlich urine test for PAS, 25 (41 per cent) gave negative reactions. All negative reactors admitted upon close questioning that they were taking PAS irregularly or not at all. Four of the 36 positive reactors admitted upon vigorous questioning that they were not taking their PAS regularly.

Breite (5) reported 90 per cent positive urine reactors for PAS among hospitalized patients while only 35.5 per cent of ambulatory patients, reacted.

Chaves (6) reported the results of the Phenistix PAS urine test in ambulatory patients for whom PAS was prescribed through the Bureau of Tuberculosis of the New York City Department of Health. Of 2,622 supposedly taking PAS, positive urine reactions for the drug were found in 60.5 per cent, with percentages ranging from 46 to 75 per cent in the various clinics. The drug was detected in the urine of only 77.8 per cent of those classified as taking PAS regularly.

Plan of Study

This report concerns drug acceptance by tuberculous patients who had been discharged from Sunshine Hospital and treated in the outpatient department in the calendar years of 1955, 1956, and 1957. During this period, dual-drug therapy with some combination of isoniazid, streptomycin, and PAS was recommended to all patients discharged from the hospital with medical advice after hospitalization for active tuberculosis in any form. Outpatient treatment was not offered those absconding or leaving the hospital against medical advice. The average hospitalization of these regularly discharged patients was nine months.

On the day of discharge, the patient and, whenever possible, a responsible member of the family was interviewed by the attending physician. During these interviews special effort was made to impress upon both the patient and his family the importance of continuing treatment without interruption. Treatment was usually recommended for a total of two years, including the time of hospitalization. A few patients who were thought to have especially poor prognoses relative to future reactivation of the disease were urged to continue the chemotherapy longer.

All of the patients receiving oral medications were given a one-month supply on the day of discharge. Those receiving streptomycin usually returned to the outpatient clinic for injections, or the drug was given at home by a public health nurse. Occasionally, a responsible member of the family was taught to give the injections. Those receiving oral drugs and those receiving streptomycin at home were required to return to the outpatient department each month for an additional one-month supply. All were encouraged to see the outpatient physician with regard to any untoward symptoms. Each patient was required to visit the outpatient department every three months for

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roentgenographic and bacteriologic studies. Patients delinquent in returning for medication or periodic check-ups were contacted by the public health nurses. The medications and re-examinations were given free of charge.

Exception to the above was made for those patients who lived in other counties. In these instances, post-sanatorium treatment was continued through their local health departments. Therefore, all of the patients included in this study resided in Kent County, Michigan. None lived at a distance greater than 30 miles. Approximately 60 per cent lived within the city in which the hospital is located. There is good bus transportation (ten minutes) from the downtown area to the hospital.

An accurate record was kept for each patient of the dates and amount of drug dispensed and of injections given. Therefore, it is possible to determine over any given number of months what portion of the prescribed dose was actually dispensed to each. For this purpose a somewhat precarious assumption must be made that, if the patient bothered to come to the outpatient department for the drugs, he was probably taking them. It is possible, though no instance is known, that the patient purchased drugs independently. It is fairly certain then that the patients were not taking more than the dispensary records indicate.

Five hundred and eighty patients were discharged from the hospital during the three-year period. The following groups were excluded from from this study: (*1*) those admitted for diagnostic study and discharged as nontuberculous or without evidence of recent active tuberculosis, and for whom antituberculous chemotherapy was not prescribed; (*2*) all patients under fifteen years of age; (*3*) those absconding or leaving the hospital against medical advice (83, or 14.3 per cent); and (*4*) those patients residing in other counties. The post-sanatorium treatment of the last-named group was managed by private physicians or other county health departments. Available records were insufficient for accurate determination of continued faithfulness to the prescribed chemotherapy. Therefore, the following report includes 264 adult patients residing within 30 miles of the hospital who had been discharged with medical staff permission during the years 1955, 1956, and 1957.

Since these patients were all given a one-month supply of the drug on discharge, the first month of post-hospital treatment is omitted from the following charts. Those who returned on three

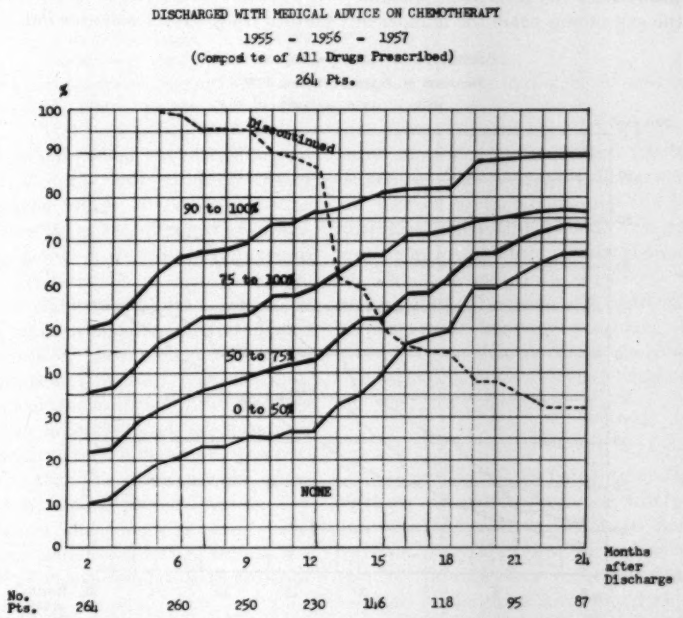

FIG. 1. The cumulative percentage of patients whose post-hospital chemotherapy was discontinued on medical staff recommendation is indicated by the descending dotted-line curve. The solid ascending curves indicate the percentage of patients who were not taking (in a composite of all of the drugs prescribed) their prescribed drugs at the given months after discharge. The actual number of patients supposedly on chemotherapy at any given time post discharge is indicated at the bottom.

occasions for a one-month supply over a six-month period were considered to be taking 50 per cent of the prescribed amount; a 2-month supply over a six-month period was considered to represent 33 per cent, et cetera.

The cumulative percentage of patients whose post-hospital chemotherapy was discontinued on medical staff recommendations is indicated in the descending curve at the upper right of figure 1. The percentages indicated in the ascending curves are based on the total number for whom chemotherapy had not been discontinued. The actual number of patients supposedly on chemotherapy at any given time post discharge is indicated at the bottom of the graph.

Results

A composite of all of the drugs prescribed is utilized in figure 1. It indicates that 10 per cent of the patients never returned for additional medication after leaving the hospital. At six and twelve months post hospitalization, 20 and 26 per cent, respectively, were no longer taking any medication. At six months, 33 per cent and, at twelve months, 43 per cent were taking none or less than 50 per cent of the prescribed amount. Although at the end of two years the number of patients whose chemotherapy had not been officially discontinued was reduced to 87 persons, 68 per cent were taking no drugs, and 73.5 per cent less than half of the prescribed amount. It should be noted that, at six and twelve months, 34 and 24.5 per cent, respectively, were taking 90 per cent or more of the prescribed dosage. A review of the records indicated that 9 sturdy individuals had rarely missed a dose in two years of outpatient treatment.

The question exists of the influence of toxic reactions and the unpleasantness of the drugs themselves on the patient's ability or inclination to continue treatment. In this study, no effort was made to determine the percentage of toxic reactions. However, no patient was discharged and prescribed a drug combination to which he had manifested intolerance while hospitalized. When toxic reactions or intolerance of significant severity did occur, the medication was discontinued or the drug combination changed by the outpatient physician. Patient acceptance of each of the three drugs was plotted in the manner described below. The results are indicated in figures

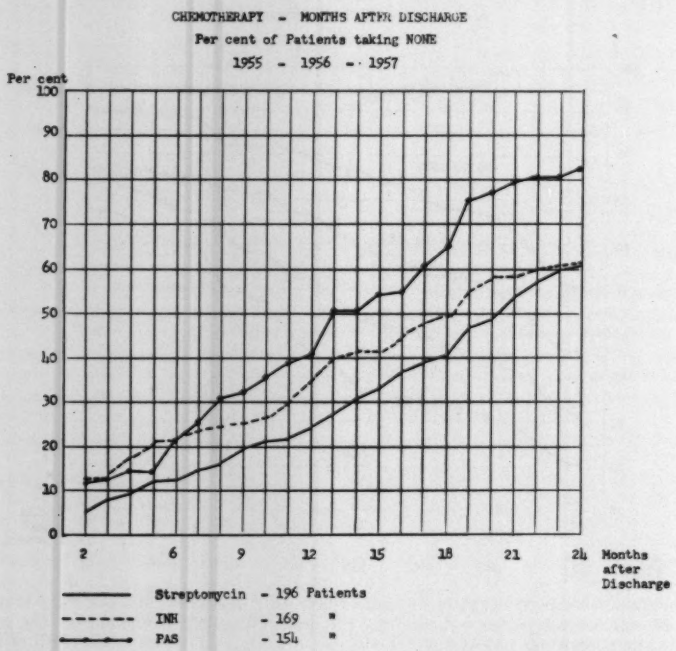

FIG. 2. Patient acceptance of chemotherapy. Percentage of patients taking no drugs at all at the given months after discharge.

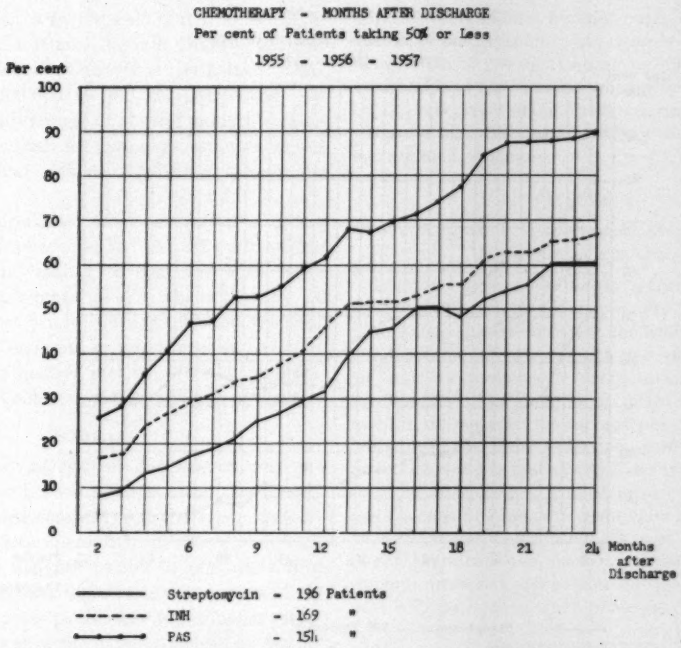

FIG. 3. Patient acceptance of chemotherapy. Percentage of patients taking 50 per cent or less of the drugs prescribed at the given months after discharge.

2, 3, and 4. It will be noted that streptomycin continuously showed the best curve of patient acceptance—ranging 18 to 30 per cent better than PAS, and 5 to 10 per cent better than isoniazid. During this period it was not customary to give isoniazid alone except in rare instances. Perhaps the poor showing with PAS can be explained in part by gastrointestinal irritation and the unpleasantness of consuming so large an amount of medication. It is noted, however, that, in spite of the inconvenience and unpleasantness of injections, the acceptance of streptomycin showed a higher curve than either PAS or isoniazid. It is believed that this is because the injections were given by second persons who tend to prevent carelessness and forgetfulness. No significant difference could be demonstrated in the acceptance of PAS when combined with either of the two other drugs.

Since hospital patients are given medicines at regular times, it is not possible to use a similar method of determining acceptance. On one day (January 16, 1960), without prior announcement or patients' knowledge of purpose, two specimens of urine were collected (10:00 a.m. and 3:00 p.m.) from each adult. These were examined for PAS or its metabolites by the ferric paper-strip test (Phenistix). Seventy-two patients were receiving PAS. Their urines showed reactions on both specimens. Fifteen patients were not receiving PAS. The urine of 3 of these showed reactions on at least one specimen. The nurses' notes indicate that all 3 had received acetylsalicylic acid or sodium salicylate within six hours of the specimen collection.

COMMENT

At the time of discharge from the hospital, virtually all patients promise firmly that they can and will take treatment at home as prescribed. Many urge their early discharge on this ground. Investigators reporting chemical tests for PAS in the urine have found no reaction in 30 to 65 per cent of ambulatory patients examined. The results shown above fall within this range, depending upon the time after hospital discharge. These results indicate a fairly regular attrition rate of 1.3 to 2.5 per cent per month

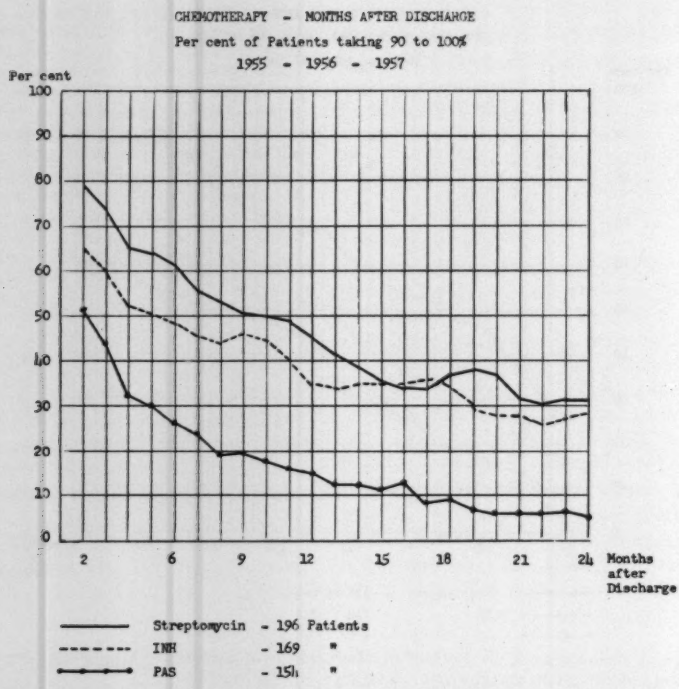

FIG. 4. Patient acceptance of chemotherapy. Percentage of patients taking 90 to 100 per cent of the drugs prescribed at the given months after discharge.

after discharge, beginning at 10 per cent. Patients taking medication in reduced dosages, or irregularly, follow approximately the same curve. The latter raise the question: at what point, with reduced or irregular dosage, does continuation of treatment become useless.

The incidence of tuberculosis is traditionally considered higher in individuals of low socioeconomic status. The area included in this study has had an effective tuberculosis educational, case finding, and hospital program for many years. Hospitalization was not compulsory until the recent past, that is, 1954. It may be assumed that the incidence of tuberculosis in the area has been affected to a relatively greater degree in the educable and cooperative segment of the population. In recent years, programs requiring chest films on admission to the county jail, rescue missions, and social hygiene clinics have been increasingly important sources of new cases. Therefore, hospitalized patients tend to represent a less cooperative group than does a mean of the general population. This is in part counter-balanced by the exclusion of those patients who leave the hospital against medical advice. The latter group contains a large percentage of alcoholic and sociopathic individuals. The 264 patients included in this study were, with 3 exceptions, those who had voluntarily entered and remained in the hospital until discharged on medical recommendation. It was known to all that patients with tubercle bacilli in the sputum could be committed to the hospital by court order. The 3 exceptions were individuals who had been so committed. One continued treatment faithfully as an outpatient. Outpatient treatment was not offered those discharged prematurely on the grounds that few could be expected to cooperate and that such practice would encourage others to leave the hospital prematurely.

Without objective evidence, it can be speculated that the persons represented in this study make up a less educated and cooperative group than is seen in the average physician's private

office. However, it is probable that they simulate very closely the run-of-the-mill patients seen in public clinics of midwestern industrial communities of 200,000 to 300,000 population. All had had the advantage of several months' education in the hospital. All had acquaintances whose tuberculosis had relapsed following inadequate treatment.

Efficient ambulatory treatment of tuberculosis necessitates emphasis on the patient's education, inclination, and ability to continue with regularity on an adequate level of chemotherapy. A close follow-up study by enthusiastic medical and nursing personnel is important and should include some objective method of estimating the amount of chemotherapy taken by the patient.

Summary

The monthly records of 264 tuberculous patients who had been discharged from the hospital with medical consent, and the recommendation of outpatient chemotherapy, were reviewed. A progressive increase in the number defecting from the prescribed regimen is demonstrated. It is noted that, after twelve months of home treatment, 26 per cent of the patients placed on ambulatory chemotherapy did not return to the clinic for renewal of their drug supply. Forty-three per cent appeared to be taking none, or less than 50 per cent, of the drugs prescribed. Only one-fourth were taking within 90 per cent of the regimen as ordered.

Sumario

La Quimioterapia para la Tuberculosis en los Enfermos Externos

Se repasaron los protocolos mensuales de 264 tuberculosos que habían sido dados de alta del hospital con el asentimiento del médico, y la recomendación de quimioterapia en calidad de enfermos externos. Se descubrió un aumento creciente en el número de los que abandonaban el régimen prescrito. Nótase que, al cabo de doce meses de tratamiento en casa, 26 por ciento de los enfermos asignados a quimioterapia ambulante no volvieron a la clínica para reponer su abasto de drogas. Un 43 por ciento parecía que no tomaban medicación, o que tomaban menos de 50 por ciento de la prescrita. Solamente la cuarta parte de los enfermos tomaba aproximadamente 90 por ciento del régimen ordenado.

Resume

Chimiothérapie de la tuberculose chez des malades de consultation externe

L'auteur a revu les fiches mensuelles de 264 malades tuberculeux qui ont été renvoyés de l'hopital avec consentement médical et à qui il a été recommandé d'appliquer une chimiothérapie en tant que malades de consultation externe. Il est montré qu'il y eut une quantité croissante du nombre de malades ne suivant pas la conduite à tenir prescrite. Il est noté que, après douze mois de traitement à la maison, 26% des malades placés sous chimiothérapie ambulatoire, ne revinrent pas à la consultation pour renouveler leur provision de médicament. Quarante trois pour cent semblèrent ne prendre aucun, ou moins de 50%, des médicaments prescrits. Seulement un quart prit à peu près 90% de l'ordonnance prescrite.

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LYSOGENY IN THE MYCOBACTERIA[1, 2]

I. The Establishment of Lysogeny

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Introduction

In the past decade one of the most fruitful areas of research for both the bacteriologist and the geneticist has been the study of the relationships between bacteria and temperate bacteriophages. From such studies it has been determined: that *Corynebacterium diphtheriae* is not toxigenic unless it is lysogenized with certain bacteriophages (1, 2); that workable phage-typing schemes in several groups of bacteria are dependent upon temperate bacteriophages (3, 4); that genetic characteristics, such as drug resistance, can be transferred from certain strains of bacteria to recipient bacteria *via* bacteriophages (5, 6); and that much information can be gained concerning host-parasite relationships at the cellular level (7, 8). In addition, it is postulated by Hewitt (9) that temperate bacteriophages exhibit a great deal of influence on bacterial evolution and epidemiology, and by Adams (10) that lysogeny in bacteria resembles in some respects the phenomenon of latency in animal and plant viruses. To quote from a review article by Lwoff (11) "Lysogeny occupies a privileged position at the crossroads of normal and pathological heredity, of genes and viruses."

While it is well known that lysogeny is widespread in bacteria, rigorous attempts by several workers (12–14) have led to the detection of only two strains of naturally lysogenic mycobacteria (14, 15). However, there are several reports on the isolation from soil of lytic mycobacteriophages that lyse a variety of saprophytic and pathogenic mycobacteria (16–18). It was believed that lysogenic mycobacteria and temperate mycobacteriophages would be useful in genetic studies of the mycobacteria. Furthermore, there have been only inconclusive reports concerning lysogeny in any of the bacteria that normally grow as intracellular parasites within their hosts (19, 20). It is conceivable that an intracellular bacterial parasite that itself is a genetic host to an intracellular phage might serve as a useful genetic tool in the study of host-parasite relationships.

With the goal of obtaining lysogenic mycobacteria to serve as tools for future experiments, a number of representative stock cultures were tested to determine if they were naturally lysogenic. An attempt was also made to establish lysogeny in phage-susceptible cultures by exposing them to lytic phages isolated from soil samples.

The present paper is a report on the successful lysogenization of a number of types of mycobacteria, including *Mycobacterium tuberculosis* H37Rv. It is also the first report of the establishment of lysogeny in any strain of bacteria by lytic phage filtrates.

Materials and Methods

Mycobacteria: Thirty-five cultures of representative slow-growing and rapid-growing mycobacteria were employed. The majority of these cultures were obtained from the stock culture collection at Olive View Hospital, and their identification is listed in table 1.

Mycobacteriophages: The identification of the seven lytic mycobacteriophages is listed in table 2. The temperate mycobacteriophage DF2 was isolated from a stock culture of *Mycobacterium smegmatis* (F2) six years after it had been exposed to an unidentified mycobacteriophage by Dr. Leroy C. McLaren (21).

Culture media: Stock cultures of mycobacteria and mycobacteria employed as inocula for semi-solid agar overlays were grown in Difco Dubos-Tween® 80 albumin medium. Mycobacteriophage preparations and mycobacteria employed in lysogenization experiments were grown in Difco

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[2] Presented in part at the annual meeting of the Society of American Bacteriologists, Chicago, Illinois, on April 29, 1958; and before the Medical Session, as part of *Section 3B*, at the annual meeting of the American Trudeau Society, Chicago, Illinois, May 26, 1959.

[3] Pre-doctoral Fellow of the American Trudeau Society.

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heart infusion broth. One per cent glycerol was added to the heart infusion broth to enhance the growth of *Mycobacterium tuberculosis* H37Rv and *M. tuberculosis* H37Ra. The plating medium was Baltimore Biological Laboratories Bordet-Gengou agar base with peptone and 1 per cent glycerol. The 3 ml. semi-solid agar overlays were composed of Difco Bordet-Gengou broth with proteose peptone, 1 per cent glycerol, and 0.8 per cent agar. Cultures were incubated at 37° C.

TABLE 1

STOCK CULTURES OF MYCOBACTERIA

Olive View Designation	Name	Strain or Source
F1	*M. smegmatis*	UCLA S1
F2	*M. smegmatis*	UCLA S1 resistant
F3	*M. smegmatis*	UCLA S2
F4	*M. smegmatis*	UCLA S3
F5	*M. smegmatis*	ATCC 361
F8	*M. phlei*	Steenken—Trudeau
F10	*M. phlei*	McLaren, P4
F13	*M. lacticola*	ATCC 9626
F21	*M. smegmatis*	ATCC 607
F24	*M. phlei*	Gordon #39
F46	*M. tuberculosis*	Isolated at Olive View Hospital
F60	*M. avium*	Mayo Clinic #3717
F61	*M. avium*	Mayo Clinic #4110
F62	*M. avium*	Mayo Clinic #4133–7
F63	*M. avium*	Mayo Clinic #4121
F66	*M. avium*	Mayo Clinic #3965
F70	*M. tuberculosis*	H37Ra—Steenken, Trudeau
F71	*M. tuberculosis*	H37Rv—Steenken, Trudeau
F80	*M. tuberculosis*	BCG, N. Y. State Dept. Health
F87	*M. smegmatis*	USA, Rome, Italy
F89	*M. phlei*	Ceppo, Rome, Italy
F95	*M. phlei*	Whittaker #2
F107	*M. fortuitum*	ATCC 6841
F116	*M. smegmatis*	ATCC 101
F119	*M. species*	ATCC 9033
F130	*M. species*	ATCC 4243
F140	*M. smegmatis*	ATCC 361
F151	*M. species*	ATCC 9819
F187	*M. species*	Hauduroy, CR5
F212	*M. phlei*	Zeman, Mph 3
F602	*M. phlei*	P. M. Tracey #2
F616	*M. tuberculosis*	H37Rv—Steenken, Trudeau
F797	*M. tuberculosis*	H37Rv—Runyon, Sunmount
F1151	*M. fortuitum*	Isolated at Olive View
—	*M. Friedmanni*	Occidental College

TABLE 2

IDENTIFICATION OF LYTIC MYCOBACTERIOPHAGES

Olive View Designation	Propagating Host	Source
D4	F21	Olive View, soil isolate
D11	F89	Penso, *Phagus phlei*
D12	F21	Penso, *Phagus rabinovitch*
D29	F21	Olive View, soil isolate
D32	F119	Olive View, soil isolate
D55a	F21	Penso, *Phagus battaglini*
D56	F21	Penso, *Phagus minetti*

Detection of mycobacteriophage activity: Phage activity was detected by the use of young Dubos-Tween 80 albumin cultures of phage-susceptible strains of mycobacteria incorporated into melted and cooled (to 45° C.) tubes of semi-solid medium and poured on the surface of agar plates. Cell-free filtrates or sectors of clones to be tested for phage activity were either incorporated into the melted and cooled semi-solid medium or spotted on the dried surface of the poured semi-solid agar layers. The plates were then incubated and observed daily for areas of lysis until the overlay inoculum exhibited abundant growth.

Ultraviolet source: The cold-quartz ultraviolet lamp employed[5] emitted 99 per cent of the light at 2,537 Å. A one-minute period of irradiation at a distance of 25 cm. killed approximately 10 per cent of the cells of a twenty-four-hour culture of *M. smegmatis* ATCC 607 (22) suspended to a depth of 1 cm. in M/15 phosphate buffer pH 7. All irradiations were performed on mycobacterial cells at a distance of 25 cm. from the lamp.

Preparation of mycobacteriophage stocks: Lytic phage stocks were prepared by inoculating 75 ml. of heart infusion broth with 0.3 ml. of a twenty-four-hour Dubos-Tween albumin culture of host cells and incubating on a Burrell wrist-action shaking machine for eight hours. Following this period of incubation, a suspension containing 10^6 phage particles and 0.75 ml. of a sterile 1 per cent solution of calcium chloride was added, and the culture was incubated for an additional eighteen hours with continued agitation. The culture was then centrifuged for fifteen minutes at 2,000 r.p.m., and the supernatant filtered through a Millipore HA filter.[6] A 2-ml. aliquot of the filtrate was plated and incubated for one week to check for sterility of the phage preparation. The remainder of the phage preparation was stored in sterile screw-capped tubes at 4° C.

Temperate phage stocks were prepared by inoculating young Dubos-Tween albumin cultures of lysogenic mycobacteria into heart infusion broth and incubating on the shaking machine.

[5] Manufactured by the R. A. Fischer Co., Glendale, California.

[6] Millipore Filter Corporation, Watertown, 72, Massachusetts.

Rapid-growing strains were incubated for three days and slow-growing strains were incubated for three weeks. The phage preparations were then centrifuged, filtered, checked for sterility, and stored in the same manner as the lytic phage perparations.

Screening of stock cultures for lysogeny: Two different methods were employed in an attempt to detect lysogeny of the thirty-five stock cultures. In the first, a modification of the Fisk method (23), each culture was inoculated into semi-solid agar overlays, and 0.05 ml. of young Dubos-Tween albumin cultures of the other test strains was spotted on it. These plates were prepared in duplicate and one set was ultraviolet-irradiated for one minute before incubation.

In the second method, the thirty-five stock strains were grouped according to growth rate, and pooled strains in each group were incubated in heart infusion broth on the shaking machine until abundant growth was obtained. At twelve-hour intervals aliquots were removed from the pools of rapid-growing strains, centrifuged, filtered through a Millipore HA filter, and 0.3 ml. of the filtrates incorporated into semi-solid overlays with each of the thirty-five stock cultures. The pools of the slow-growing cultures were tested in a similar manner every seven days. In addition, the following modifications of the above technique were performed:

(*a*) After twenty-four-hour incubation on the shaker, the pooled cultures were centrifuged and the cells resuspended to one-half of the original volume in M/15 phosphate buffer. The cells were then ultraviolet-irradiated for one minute, centrifuged, and resuspended in the original volume of fresh heart infusion broth and returned to the shaker for additional incubation.

(*b*) A final concentration of 0.01 gm. per ml. of ethylene-diaminetetra-acetic acid (a calcium-sequestering agent) was added to the broth at the time of inoculation, and calcium chloride was added to the filtered aliquots to a final concentration of 0.01 gm. per ml. just before they were tested for phage activity. Calcium is a necessary adsorption factor in many phage-host systems and is present in small quantities in heart infusion broth. The above modification was performed to remove the calcium in an attempt to inhibit the adsorption of any phage that might be liberated in the pooled cultures to the cells present in the cultures.

(*c*) Streptomycin sulfate at the rate of 0.001 gm. per ml. was added every twelve hours for three days, and the cultures were incubated for an additional two days. The streptomycin-treated cultures were then centrifuged, the cells resuspended in fresh heart infusion broth, and returned to the shaker for additional incubation until luxuriant growth appeared or until it was apparent that the cells had been killed by the streptomycin. This attempt was made to produce streptomycin-resistant cultures, for it was demonstrated by Ferguson and associates (3) that some strains of *Salmonella* liberated temperate phage only after the cells had become streptomycin resistant. All possible combinations of methods (*a*), (*b*), and (*c*) were performed.

Preparation of mycobacteriophage antisera: Five-milliliter aliquots of stock phage preparations were inoculated subcutaneously into albino rabbits twice a week for six weeks. The titers of the lytic phage stocks were approximately 10^9 per ml. and of the temperate phage stocks approximately 10^8 per ml. The animals were then trial bled and the potencies of the antisera determined by the *k*-value technique (24). Those animals whose sera had *k*-values greater than 25 were bled by cardiac puncture, and the sera were preserved by freezing. Those animals whose sera had *k*-values less than 25 at six weeks were given further injections, and the sera were harvested when *k*-values exceeded 25.

Establishment of lysogeny: The method employed was based on the techniques of Lieb (25) and of Bertani and Nice (26). Rapid-growing mycobacteria to be employed in lysogenization experiments were incubated on the shaking machine for twenty-four hours and slow-growing mycobacteria were incubated for ten days. Cell numbers were estimated by examination in a Petroff-Hauser chamber, and phage preparations were added to give phage-to-cell ratios of 0.5, 1.0, and 5.0. Calcium chloride was added to the phage-host combinations to a final concentration of 0.01 gm. per ml. The mixtures were incubated at 37° C. for fifteen minutes and refrigerated for two hours. The mixtures were then centrifuged and the cells resuspended in heart infusion broth that contained sufficient antisera to inactivate at least 5,000 homologous phage particles. After an additional thirty-minute incubation at 37° C., aliquots estimated to contain approximately 200 surviving cells were spread on the surface of 10 agar plates. The plates inoculated with rapid-growing strains were incubated for one week and those inoculated with slow-growing strains were incubated for six weeks.

Twenty colonies from plates of each phage-host mixture were streaked on agar plates and incubated until good growth appeared. Two isolated clones obtained from each of the twenty colonies were tested for phage production by placing a portion of the clone on the surface of a semi-solid agar plate seeded with a phage-susceptible strain of mycobacteria. If phage activity was detected after incubation of the plates, another portion of each of the clones was inoculated into a tube of heart infusion broth containing appropriate phage antiserum and incubated for forty-eight hours. Loopfuls of the broth cultures were then streaked on agar plates and, after incubation, isolated colonies were again tested for phage production by the above method. Those clones that exhibited phage production were

tested for the stability of the lysogenic condition by making three successive single colony transfers.

If isolated clones from the third transfer still exhibited phage production, they were tested for their resistance to lysis by the phage they produced. This was performed as follows: Dubos-Tween albumin cultures and temperate phage stocks were prepared from the clones in question. A semi-solid agar layer was inoculated with the young Dubos culture, poured on an agar plate, and allowed to dry. A drop of the homologous phage stock containing 10^3 to 10^4 phage particles was placed on the surface of the agar layer. The plates were then incubated and observed for phage activity. Cultures that produced phage and exhibited resistance to the phage they produced were considered lysogenic (11).

Animal virulence tests: Swiss Webster mice weighing 20 to 22 gm. and albino guinea pigs weighing 300 to 350 gm. were inoculated intraperitoneally with 0.2 ml. of four-day-old Dubos-Tween albumin cultures of rapid-growing mycobacteria. Guinea pigs were inoculated subcutaneously with 0.2 ml. of fourteen-day-old Dubos cultures of *M. tuberculosis* diluted 1:100 in sterile saline.

Cytochemical tests: The combined neutral red and cording test of Wayne was employed (27).

Niacin test: A modification of the method of Konno and associates (28) was employed. Cultures with abundant growth were overlayered with 1 ml. of 4 per cent aniline in absolute ethanol and followed in two to three minutes with 1 ml. of a 10 per cent aqueous solution of cyanogen bromide. The cultures and fluid were observed from one to five minutes for the formation of a canary yellow color.

Catalase and peroxidase tests: A modification of the method of Tirunarayanan and Vischer (29) was employed. The cultures were overlayered with 1 ml. of 0.2 per cent catechol in 3 per cent hydrogen dioxide and 3 per cent Tween 80. The colonies were observed within a few minutes for the emission of bubbles of oxygen and again in an hour to determine whether the colonies exhibited a brownish-black discoloration.

Results

Screening of stock cultures for lysogeny: One of the thirty-five stock cultures screened for lysogeny exhibited phage production in all of the tests described in the preceding section. This culture, *Mycobacterium smegmatis* (F2), was exposed to an unidentified mycobacteriophage in 1951. It was maintained on egg medium in the stock culture collection at Olive View Hospital from 1952 until the present experiments were begun in 1957. None of the other thirty-four cultures exhibited phage production by any of the described screening methods, and none of them had been exposed to phage in previous laboratory experiments.

TABLE 3

Results of Lysogenization Experiments

Hosts	Mycobacteriophages	
	Lysogeny Established	Lysogeny Not Established
M. tuberculosis		
F46	D32, D32(F21)	D29
F70(H37Ra)	D32, D32(F21), D32(F616)	D29
F71(H37Rv)	D32, D32(F21)	D29
F80(BCG)	D29(F21), D32(F21), D32(F616)	D29, D32
F616(H37Rv)	D32, D32(F21)	D29
M. smegmatis		
F21(ATCC 607)	D4, D12, D29, D32, D55, D56, DF2, DF2(F21), D29(F21), D32(F21), D32(F616)	
F21(SM_1)	D29, D32, D29(F21), D32(F21), D32(F616)	
F21(PM_1)	D29, D32, D29(F21), D32(F21), D32(F616)	
F116	D32, DF2(F21), D29(F21), D32(F21), D32(F616)	D29
M. phlei		
F24	D29, D32(F21)	D11
M. fortuitum		
F107	D32, D29(F21), D32(F21)	D29
F1151	D32, DF2(F21), D32(F21)	D29
M. species		
F119	D29, D32, DF2, DF2(F21), D29(F21), D32(F21), D32(F119), D32(F616)	
F151	D29, D32	

Establishment of lysogeny: The first Mycobacterium employed in attempts to establish lysogeny in the stock cultures was *M. smegmatis* ATCC 607 (F21) and success with that host led to an extension of lysogenization experiments to other hosts. The results of the lysogenization experiments are summarized in table 3 and indicate that lysogeny can be established in many types of mycobacteria by a variety of

mycobacteriophages. The experimental evidence indicates a slightly higher rate of lysogenization with 5 phage particles per cell than with 0.5 or 1.0. Preliminary studies have demonstrated that in some strains lysogeny can be established without refrigeration of the phage-host mixture immediately after the initial adsorption period.

The cultures lysogenized during the course of this study are designated by the stock culture number of the Mycobacterium followed in parenthesis by the stock number of the phage to which it was exposed, *e.g.*, the lysogenic culture that results after exposure of *Mycobacterium* F21 to a filtrate of lytic phage D29 is designated as F21(D29). The temperate phages that are released from the lysogenic mycobacteria are designated by the stock number of the phage followed in parenthesis by the stock number of the Mycobacterium in which lysogeny was established, *e.g.*, the temperate phage released after *Mycobacterium* F21 was lysogenized by exposure to a filtrate of lytic phage D29 is designated as D29(F21).

There is a wide variation in the ability of lytic *vs.* temperate mycobacteriophages to establish lysogeny under the conditions employed in these experiments. The exposure of ATCC 607 to lytic phages killed at least 95 per cent of the cells, and the majority of the survivors exhibited no detectable phage production. The rate of lysogenization by filtrates of lytic phage D32 was approximately 10^{-6} and the rate of lysogenization by the other lytic phages was less than 10^{-8}. However, when ATCC 607 was exposed to

FIG. 1. Rough colonial morphology of ATCC 607.

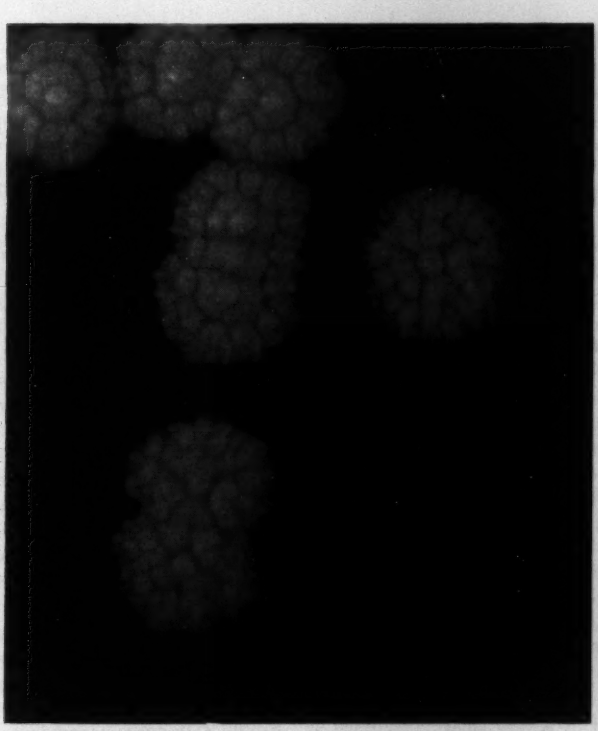

FIG. 2. Smooth colonial morphology of ATCC 607.

temperate phages, at least 20 per cent of the cells survived, and the approximate rate of lysogenization ranged from 0.1 per cent to 30 per cent. The more limited results with other host strains indicate the same general trend of extremely low rates of survival, with little or no lysogenization after exposure to lytic phage, and relatively high rates of survival and lysogenization after exposure to temperate phage.

Further tests were performed to check the stability of the lysogenic condition of these cultures. Twenty cultures were selected that included each of the host strains listed in table 3. Approximately half of them were lysogenized with lytic phage filtrates and half of them were lysogenized with temperate phage filtrates. Each of these strains was submitted to ten consecutive single colony transfers, and five isolated clones from each transfer in the series were tested for phage production by spotting them on indicator strains. Phage production was detected in 991 of the 1,000 clones tested. These same twenty cultures were also submitted to five consecutive transfers in tubes of heart infusion broth containing sufficient phage antisera to inactivate at least 5,000 homologous phage particles. Each serum tube inoculated with slow-growing strains was incubated one week before transfer, and each serum tube inoculated with rapid-growing strains was incubated for two days. From the fifth serum tube of each strain, a loopful of broth was streaked on an agar plate, and after incubation ten isolated clones from each plate were tested for phage production. Phage production was detected in 195 of the 200 clones tested.

The occurrence of colonial variants: It was noted early in the lysogenization experiments that most of the cells of ATCC 607 that survived exposure to lytic phages exhibited a colonial morphology that was smoother in appearance than the colonial morphology of the original stock ATCC 607. Tests on approximately 1,500 of these colonies revealed that most of them were phage resistant but did not produce phage. However, 194 of the 200 isolates of ATCC 607 that *were* lysogenized by exposure to lytic phage

filtrates did exhibit this smooth colonial morphology. The six strains that did not exhibit smooth colonial morphology were initially exposed to the phage in the presence of the homologous phage antiserum. Only rarely were smooth colonial variants observed after exposure of ATCC 607 to temperate phage preparations.

In an attempt to elucidate the origin of the smooth colonial variants, 10^5 cells of ATCC 607 were spread on the surface of agar plates in the absence of phage. After incubation, two of the smooth colonial variants were observed and isolated. Phage typing of these two cultures proved them to be resistant to most of the lytic and temperate phage stocks. One of these cultures ($F21SM_1$) and one of the phage-resistant, apparently nonlysogenic, smooth colony types derived from the exposure of ATCC 607 to lytic phage D32 ($F21PM_1$) were employed in lysogenization experiments as listed in table 3. The results show that these cells are capable of being lysogenized and that all of the lysogenized cultures derived from them exhibit smooth colonial morphology. The rough colonial morphology typical of stock ATCC 607 and of ATCC 607 lysogenized by temperate phage is illustrated in figure 1. The smooth colonial morphology exhibited by a very small percentage of cells in stock ATCC 607 and by the majority of cells of ATCC 607 following exposure to lytic phage may be noted in figure 2. Occasionally colonial variants of other strains of mycobacteria have appeared after exposure to lytic phage. However, these have been detected too infrequently and sporadically to indicate a correlation between colonial morphology and lysogeny or phage resistance.

TABLE 4

MYCOBACTERIA EMPLOYED IN IN VITRO CHARACTERIZATION TESTS

ATCC 607	H37Rv
F21*	F616*
F21(DF2)*	F616(D32)*
F21(D29)*	F616(D32-F21)*
F21(D32)*	BCG
F21(D32-F616)*	F80
H37Ra	F80(D32-F21)
F70	
F70(D32)	

* Also employed in guinea pig virulence tests

TABLE 5

STRAINS OF M. FORTUITUM EMPLOYED IN MOUSE VIRULENCE TESTS

F107	F1151
F107(D32)	F1151(D29-F21)
F107(D29-F21)	F1151(D32)
	F1151(D32-F119)

Characterization of representative lysogenic mycobacteria: The cultures listed in table 4 were subjected to the following tests: catalase, peroxidase, niacin, neutral red, cording, and acid-fast stain. The reactions exhibited by the lysogenic cultures were identical to those of the nonlysogenic stock cultures from which they were derived.

Animal virulence tests: Each of the cultures listed in table 4 and marked with an asterisk was inoculated into three tuberculin-negative guinea pigs. Guinea pigs inoculated with *M. tuberculosis* H37Rv and its lysogenic derivatives were tuberculin positive at five weeks, and all died within nine weeks after inoculation. Autopsy revealed gross pathology typical of progressive tuberculosis, and there were no detectable differences between the groups of guinea pigs inoculated with lysogenic or nonlysogenic tubercle bacilli. Cultures recovered from lung and spleen tissues revealed that the lysogenic tubercle bacilli remained lysogenic through animal passage.

Guinea pigs inoculated with *M. smegmatis* ATCC 607 and its lysogenic derivatives were tuberculin negative at four and nine weeks. The animals were sacrificed at ten weeks, and observation at autopsy and culture of spleen, liver, and lung tissues disclosed no sign of infection.

Each of the cultures of *M. fortuitum* listed in table 5 was inoculated into three Swiss Webster mice. Two of the mice died at five weeks and the remainder were sacrificed at six weeks. Autopsy revealed liver, spleen, and kidney involvement in approximately 70 per cent of the mice, and there were no detectable differences between the groups of mice inoculated with lysogenic or nonlysogenic bacilli. Cultures were recovered from the spleen tissues of three mice infected with three different strains of lysogenic cells, and subsequent tests revealed that the cultures remained lysogenic through animal passage.

Plaque morphology: All seven of the lytic mycobacteriophages employed in this study

produced clear plaques when plated with their propagating hosts in the lytic cycle. However, in all cases, the temperate phages released from lysogenic cultures produced turbid plaques when again plated with their original propagating hosts. The turbidity of the plaques is due to the lysogenization of some of the host cells by the temperate phage.

Discussion

Attempts to detect the existence of naturally lysogenic strains among the thirty-five stock cultures revealed phage production by only the one culture known to have been exposed to phage in previous laboratory experiments. Despite the fact that the rather exhaustive screening program did not detect phage production in the other cultures, it cannot be claimed that they are not lysogenic. It may be that little or no phage was produced under the conditions of the experiments, or that the experimental conditions employed did not permit the detection of phage.

The experimental evidence indicates that lysogeny can be established in many types of mycobacteria by exposure to a variety of lytic or temperate mycobacteriophages. The fact that lysogeny was not detected in some of the host-phage combinations tested does not necessarily imply that lysogeny cannot be or was not established in those systems. It may be that the experimental conditions did not favor the establishment of lysogeny, that too low a rate of lysogeny was established to be detected by the small number of colonies that were examined, or possibly that the host cells were lysogenized so stably that their ability to produce phage was not detected by the experimental procedures employed.

While there have been a number of reports in the literature of the isolation of lytic phage mutants from temperate phage stocks (30, 31), there have been no reports of the establishment of lysogeny by lytic phage filtrates. There are several possible explanations for this seeming anomaly, and it is hoped that current experiments will clarify this problem.

White and Knight (32) reported the occurrence of phage-resistant survivors after exposure of *M. smegmatis* ATCC 607 to lytic phage D29. These survivors formed colonies that were smoother in appearance than the phage-susceptible strains from which they were derived. The same results were obtained in this laboratory and, in addition, phage-resistant, smooth colonies were obtained after exposure of ATCC 607 to each of the five other lytic phages employed in the lysogenization experiments. Studies revealed that: more than 95 per cent of the survivors following infection with lytic phage produced smooth-type colonies; all of the smooth colonies are relatively resistant to lytic phage and in addition, some of them are lysogenic; a very small number of phage-resistant cells that produce smooth colonies spontaneously appear in stock cultures of ATCC 607; both the smooth colonies that appear in stock cultures and the smooth colonies that appear after phage infection can be lysogenized and retain their smooth colonial morphology; stock ATCC 607 can be lysogenized with lytic phage in the presence of homologous antiserum and retain its rough colonial morphology; less than 0.01 per cent of the cells of ATCC 607 exposed to temperate phage produce smooth colonies. It is believed that this is sufficient evidence to postulate that the cells that produce smooth colonies arise by spontaneous mutation in the stock culture and that their phage resistance permits them to predominate after infection of stock ATCC 607 with lytic phage. They may or may not be lysogenized during this infection, and cells of either colonial type can be lysogenized by lytic or temperate phage under the appropriate experimental conditions.

Stinebring and Braun (20) have recently reported phage-resistant colonial variants of *Brucella* after exposure to lytic brucellaphage, and suggest that mutants of this type are present in low percentages in practically all *Brucella* cultures. These investigators suggest that those phage-resistant colonial mutants as well as a bacteriocin-like material may be responsible for some of the difficulty in interpreting reports of phage activity in the brucellae.

The results of a preliminary study on a variety of tests frequently employed for the classification of mycobacteria demonstrate that lysogenization has not altered the reactions of the mycobacteria to these tests. The literature indicates that only a few varieties of temperate phage cause marked alterations of host cells, and a further search for such changes will be made.

Summary

Natural lysogeny could not be demonstrated in thirty-four stock cultures of representative saprophytic and pathogenic mycobacteria with no previous history of laboratory exposure to mycobacteriophage. It has been demonstrated that a number of these strains can be lysogenized by exposure to lytic or temperate mycobacteriophage under laboratory conditions. Phage-induced alterations in cultural or biochemical reactions were not detected among the lysogenized strains. Lysogenic cultures of *M. tuberculosis* H37Rv and *M. fortuitum* are virulent and remain lysogenic through animal passage.

Sumario

La Lisogenia en las Bacterias: I. El Establecimiento de la Lisogenia

No pudo descubrirse lisogenia natural en 34 cultivos stock de típicas micobacterias saprofitas y patógenas sin historia de exposición a micobacteriófagos en el laboratorio. Se ha demostrado que puede lisogenizarse a varias de esas cepas por la exposición a micobacteriófagos líticos o templados en condiciones de laboratorio. Entre las cepas lisogenizadas, no se descubrieron alteraciones provocadas por los fagos en las reacciones culturales o bioquímicas. Los cultivos lisógenos del *M. tuberculosis* H37Rv y del *M. fortuitum* son virulentos y continúan siendo lisógenos en sus pases a través de animales.

Resume

Lysogénie chez les Mycobactéries: I. l'Establissement de la lysogénie

Une lysogénie naturelle ne put être démontrée dans 34 cultures d'un assortiment de bactéries de types saprophyte et pathogène, sans anamnèse d'exposition au mycobactériophage en laboratoire. On a démontré qu'un nombre de ces souches peut être lysogénisé en les exposant à un bactériophage lytique ou "tempéré", dans des conditions de laboratoire. On ne décela pas de modifications induites par le bactériophage dans les réactions de culture ou les réactions biochimiques, parmi les souches lysogénisées. Des cultures lysogénisées de *M. tuberculosis* H37Rv et *M. fortuitum* sont virulentes et restent lysogéniques à travers les passages sur animaux.

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Case Report

WEGENER'S SYNDROME[1]

Case with Unusual Features

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INTRODUCTION

The decline in the incidence of tuberculosis and, particularly, the control of the individual case by chemotherapy have caused several other chronic respiratory diseases to emerge to recognition. One of these, perhaps the most bizarre of all, is Wegener's syndrome, which became known in 1936 as a rare variant of periarteritis nodosa. Because it was obscured as a manifestation of a larger entity, case reports have appeared under a variety of titles and the number has multiplied only in recent years. Reviews of the literature are now available (1) and one will not be attempted here. The chief features of the disease will be outlined and a case will be described that differs somewhat from the usual.

Wegener's description of the syndrome with which his name has become justly associated was based upon three cases, and little of importance has been added since (2). There was an initial rhinitis followed by systemic symptoms, fever, erythematous and pustular eruptions, arthralgia, leukocytosis (often with eosinophilia), anemia, and finally death from renal failure. Wegener's first patient had a "saddle nose" deformity and another a gumma-like lesion of the nasal septum with perforation. One patient had an ulcerative stomatitis. The disease ran its course in four to seven months.

Pathologically, there were diffuse granulomatous, necrotizing lesions in the lungs, liver, and kidney, together with widespread segmental arterial lesions that resembled those of periarteritis nodosa. The nephritis, apart from the granulomas, duplicated "focal nephritis" of Fahr (3). Wegener termed it granulomatous nephritis. The arterial lesions categorized the disease, and the writer of a recent extensive study (4) consequently speaks of "polyarteritis nodosa" with and without pulmonary lesions but considers at the same time the possibility that the latter is a separate entity.

The only pathogenic agent that has been consistently isolated is *Staphylococcus pyogenes*.

The introduction of chemotherapy and corticosteroids has not altered the fatal course of the disease in any essential manner, only prolonging life, perhaps, in some cases.

CASE REPORT

J. S., a 48-year-old white female patient, who was never ill in her life, developed a sinusitis in January, 1959. Her nose was blocked and she had difficulty in breathing. Later on, she developed a cough. She was hospitalized in a general hospital with these symptoms in April, 1959, treated with different antimicrobials systemically and with suctions locally. This treatment failed to improve the sinusitis; moreover, a destruction of the nasal cartilage resulted in a "saddle nose."

A chest roentgenogram (figure 1) revealed a lesion in the left upper lobe which was thought to be either a lung abscess or, more likely, a bronchogenic carcinoma. Bronchoscopy performed on April 17, 1959, revealed the left stem bronchus to be slightly hyperemic in its distal portion, and there was suspicion of extrinsic pressure on the segmental bronchial orifices suggesting an extrabronchial tumor. A moderate amount of mucopurulent secretion, slightly tinged with blood, was aspirated and washings were obtained.

Bacteriologic studies revealed *Staphylococcus aureus*, coagulase positive, resistant to tetracycline, penicillin, chlortetracycline, and susceptible to erythromycin and chloramphenicol. On May 6, 1959, a left upper lobectomy was performed, the presumptive preoperative diagnosis being bronchogenic carcinoma. Pathologic examination of the specimen revealed chronic, granulomatous inflammation consistent with fibrocaseous tuberculosis. The lung lesion was described as an ill-defined, fibrocaseous multiloculated cystic mass, measuring 3.2 cm. in greatest diameter, communicating with the main stem bronchus to this region. The bronchus was thickened and partially occluded by material similar to that composing the mass. Microscopic sections revealed large areas of

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FIG. 1. Opacity in lower lobe of left lung in which areas of rarefaction are shown.

caseous necrosis and marked fibrosis; multinucleated cells of the Langhans' type were prominent. Many areas of necrosis contained large numbers of polymorphonuclear leukocytes.

An erythrocyte count in April was 4,410,000 per cu. mm.; hemoglobin was 12.9; the leukocyte count, 13,100 per cu. mm., with 75 per cent neutrophils (filamented, 70 per cent; nonfilamented, 5 per cent); eosinophils, 6; lymphocytes, 16; monocytes, 3. The urinalysis was albumin negative; sugar negative. Occasional leukocytes and erythrocytes were found on microscopic examination. From the nasal secretions, *Staphylococcus aureus* (coagulase positive) was cultured. The above findings were taken from copies of the records of the hospital where the patient was first hospitalized from April 2 until May 15. The diagnoses on discharge were: pulmonary tuberculosis, chronic sinusitis, possible tuberculosis of nasal cartilage.

Two weeks later, the patient was admitted to the Herman Kiefer Hospital, tuberculosis division, with the chief complaint of extreme weakness and very troublesome crusting and blocking of her nose. She was acutely ill, febrile, very pale, with the main physical finding of a saddle nose. The nasal mucosa was atrophic and covered with dry bloody crusts. The surgical wound from the four-week-old lobectomy was well healed. The lung fields were clear to percussion and auscultation. The heart was not enlarged. Heart rhythm was regular, no murmurs were heard. Blood pressure was 116 systolic, 80 diastolic. There were no other positive physical findings.

The chest roentgenogram revealed no parenchymal infiltrations, no postoperative space. The left sixth rib was missing. Laboratory findings on admission were: leukocyte count, 12,500 per cu. mm.; erythrocyte count, 2,820,000 per cu. mm.; hemoglobin, 8.5 gm. The differential leukocyte count revealed 82 polymorphonuclears, 17 lymphocytes, 1 eosinophil; hematocrit, 29 per cent; reticulocytes, 0.7 per cent. Urinalysis on admission showed 1-plus albuminuria, few leukocytes, few epithelial cells. The tuberculin skin reaction to intermediate-strength PPD was plus 12 mm.; serologic tests for syphilis (Kahn, Kolmer, and RPCF) were negative. The sputum culture again revealed coagulase-positive *Staphylococcus aureus* resistant to all tested antimicrobials except novobiocin, triacetyloleandomycin-glucosamine, and erythromycin.

Course in hospital: Antituberculous chemotherapy with PAS and isoniazid was instituted on admission as well as with oleandomycin and novobiocin, since the presenting clinical picture was taken for a septicemia. The spiking temperatures up to 104°F. especially seemed to justify massive antimicrobial coverage. However, blood cultures, four in all, showed no growth. Within the first two weeks, there was subjective improvement, and the temperatures gradually decreased to normal levels. On the sixteenth hospital day, pustules on both legs appeared. These were surrounded by petechiae, suggesting septic embolic phenomena. Next day, severe pain in the left eye developed, and both conjunctivae were injected. The condition was diagnosed by the consulting ophthalmologist as uveitic glaucoma, secondary to keratoconjunctivitis. During the last few days, arthalgia and an asthma-like attack of wheezing and shortness of breath had developed. The urinary output diminished rapidly. Blood urea nitrogen rose to 136 mg. per 100 ml., and the patient died in uremia on the twenty-second hospital day.

Laboratory findings: The laboratory findings during the three-week course in the hospital were as follows (only the contributory findings are listed): The leukocyte count ranged between 9,500 and 13,800 per cu. mm., with hemoglobin 7 to 9.8 gm. The erythrocyte count was 2,780,000 to 3,200,000 per cu. mm.; the differential leukocyte count showed no shift, but the eosinophils increased from 1 to 8. Urinalysis revealed increasing albuminuria to 2-plus, hematuria, and pyuria. Three lupus erythematosus preparations were negative; the erythrocyte sedimentation rate was 68 cu. mm.; an electrocardiogram was within normal limits.

Three clinical diagnoses were entertained: (*1*) staphylococcic septicemia with acute glomerulonephritis; (*2*) a collagen disease, such as periarteritis nodosa; and (*3*) Hodgkin's disease.

Post-mortem examination (positive findings only): The body was that of a rather tall white female apparently in her forties. Her nutritional state was good. There was a "saddle deformity" of the nose apparently due to sinking of the cartilage. The nasal bones were intact. No evidence of active inflammation was seen. Explored from above, no exudate or bony change was encountered in the nasal passages. Whatever had caused the deformity seemed to have healed. The skin was waxy white, and there was a suggestion of edema. The main incision revealed wet tissues but no consider-

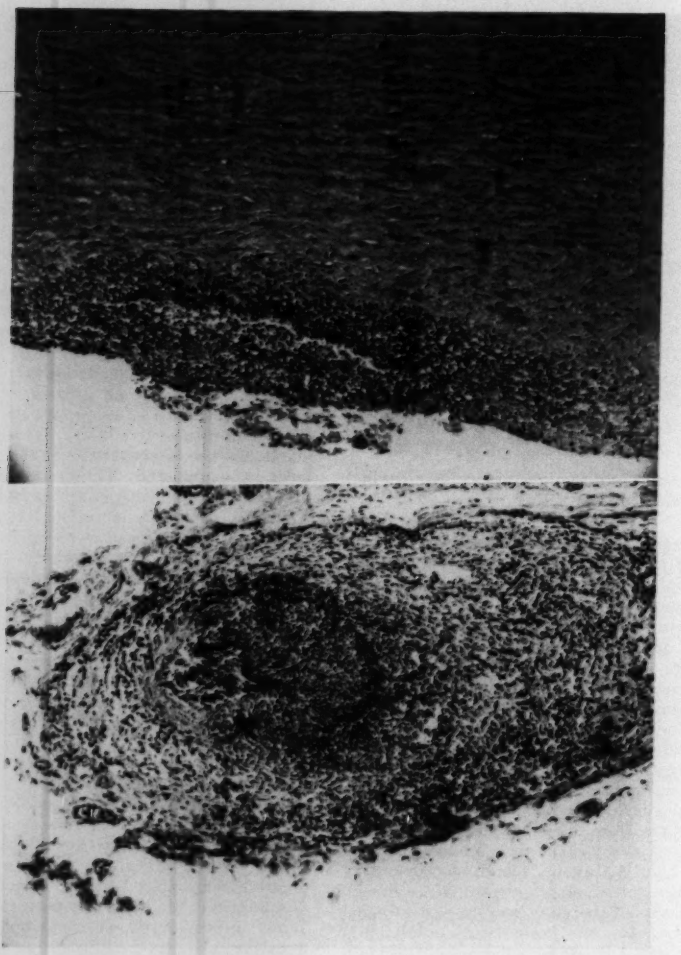

FIG. 2. (Upper). Acute focal endarteritis, aorta.
FIG. 3. (Lower). Acute necrotizing arteritis, mesentery.

able excess of fluid in the serous cavities. The heart weighed 370 gm., the hypertrophy involving the left ventricle. The lungs contained a small excess of blood and fluid, especially in the dependent portions, and there was red consolidation in the lower lobe left, particularly in segments 9 and 10. The kidneys were large, the capsules tense. The subcapsular surfaces were smooth; surfaces made by cutting were quite red, most marked in the medullae; the normal cortical markings were obscured and there were a few scattered petechial hemorrhages in the parenchyma and in the mucosa of the pelves.

Because of the clinical suspicion of periarteritis nodosa, small vessels were carefully inspected, particularly the mesenteric artery, in which three minute white places were found close to the jejunum. Also a few similar lesions were found in the vessels of the circle of Willis. The microscopic examination revealed bronchopneumonia, subacute glomerulonephritis and a very few foci of phlebitis and arteritis. *B. proteus*, *E. coli*, and diphtheroids were cultured from the bronchi; no staphylococci were isolated.

Diagnosis: The preliminary pathologic diagnosis was nephritis and terminal pneumonia as a sequel to a severe staphylococcic infection, at some stage septicemic. It was only after the complete

history, original roentgenograms, and material from the resected pulmonary lesion became available that the true nature of the condition became apparent. The identifying lesions of Wegener's syndrome herewith follow:

Arterial lesions: An acute focal necrotizing and exudative lesion occurred in the intima of the aorta. The cells of the exudate seemed to be degenerating neutrophils (figure 2). There were focal necrotizing and exudative lesions of mesenteric arteries (figure 3). The structures noted grossly in the cerebral arteries could not be identified in sections except as plaques of atherosclerosis.

Phlebitis: Thrombosis with early organization and canalization occurred in a mesenteric vein. In the same region a vein was found that showed proliferation and piling up of endothelium without leukocytic infiltration.

Renal lesions: The renal lesions consisted of necrosis of capillary loops with the appearance of thrombosis; proliferation of epithelial

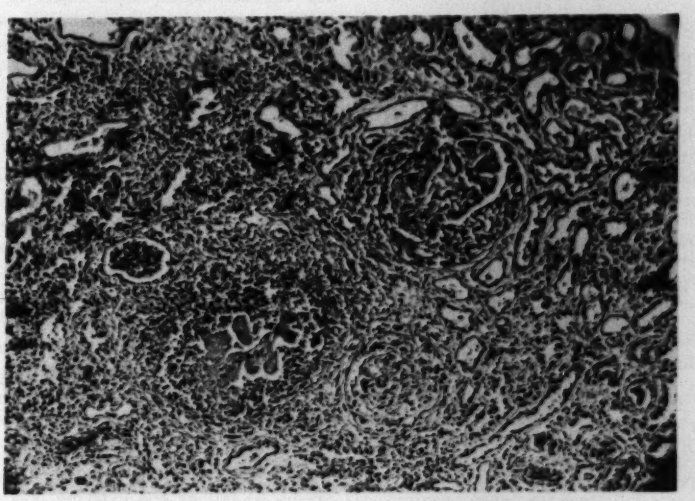

FIG. 4. Kidney: proliferative glomerulitis; interstitial infiltration and fibrosis.

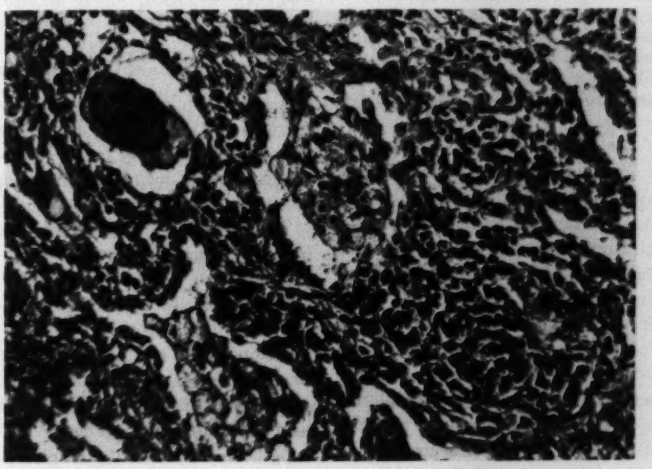

FIG. 5. Chronic pneumonia with foamy macrophages and a giant cell in the alveoli; interstitial infiltration and fibrosis.

cells and endothelial cells of capillary loops, and slight thickening of basement membranes, adhesions between glomerular tufts and Bowman's capsule; "crescents" in Bowman's capsules; and, finally, there was a rather diffuse interstitial lymphocytic infiltration and fibrosis of the stroma (figure 4). No active lesions of vessels were noted apart from those in the glomerular capillaries. Hyalinized glomeruli seemed to be secondary to a degree of arteriolonephrosclerosis unrelated probably to the acute and subacute lesions.

Lung: Sections of the mass resected at the time of the previous hospitalization had histologic features that were entirely compatible with treated tuberculosis but would hardly be expected in a recently acquired untreated infection. The mass that had been seen with the bronchoscope was composed of cellular, vascular, fibrous tissue. In other places necrotic lesions were rich in leukocytes to the point of almost constituting abscesses. These were limited by rather mature fibrous tissue which frequently contained Langhans' giant cells. At the periphery there was a monocytic and granulomatous pneumonia and all the stages of regression with organization and healing. In places there were considerable numbers of macrophages with foamy cytoplasm (figure 5). Obliterative vasculitis which was associated with the lesion was of a character no different from that seen in tuberculosis. No vascular changes were found that seemed to be intrinsic and independent of the granuloma.

Kidneys: In the medulla of one kidney were minute areas of necrosis surrounded by a vague palisade of epithelioid cells, and in a glomerulus a distinct epithelioid tubercle-like lesion was found.

Liver: A focal lesion in the liver was composed of epithelioid cells and fibroblasts, perhaps centered about a blood vessel.

Discussion

Clinically, most cases of Wegener's syndrome begin with a protracted nasal inflammation which is followed in a variable length of time by systemic symptoms which suggest a septic state. *Staphylococcus aureus* has been isolated from the nasal lesions quite consistently.[2]

Reasoning from this clinical sequence and the character of the lesions found at autopsy, Wegener suggested that the latter were a manifestation of allergy to the products of staphylococci. This is a plausible explanation of the pathogenesis of the disease, but it seems not to impress those who have devoted themselves to the study of the biology of Staphylococcus. Rigdon in a series of papers between 1935 and 1945 (5, 6) was satisfied that all staphylococcic lesions could be accounted for by the direct action of toxins, and recently Elek (7) has dissented from theories that assign allergic tissue reactions to staphylococci products as a cause of disease. It appears, however, that staphylococci at least play an important role as a secondary invader in this syndrome.

Whatever may be the underlying etiology, the basic lesions of Wegener's syndrome are of a character that is thought to be due to antibody-antigen complexes acting at the tissue level. Germuth (8) has duplicated exactly the vascular and granulomatous lesions experimentally by means of foreign protein, and a number of workers have reproduced the nephritis by similar means (9). Germuth has shown that the results of this kind of experiment hinge upon the quantitative relations between the antigen and antibody and, in addition, the element of time during which the resulting complexes are made to act (10).

The literature reveals a series of conditions which range from classic periarteritis nodosa to a mixture of periarteritis and granulomatous lesions, the latter being occasionally especially conspicuous. At least one reported case was finally classified as sarcoidosis. The present case seems to fall at the extreme end of the scale, with the granulomatous lesions dominant and the vascular lesions few and only demonstrable by a careful search.

[2] In an English series, Streptococcus figured prominently (4).

Summary

A case is reported which presented the essential features of Wegener's syndrome: an initial nasal infection followed by fever, leukocytosis, eosinophilia, anemia, cutaneous eruptions, arthropathy, and terminal nephritis.

The general condition of the patient, which was initially thought to be sepsis caused by *Staphylococcus aureus*, continued little altered by therapy although the staphylococci disappeared from the respiratory secretions.

At operation and autopsy, granulomatous and vascular lesions were found, the latter resembling periarteritis nodosa. A "saddle nose" was an unusual but characteristic complication. Of especial interest were: a single pulmonary granuloma (rather than diffuse lesions), which simulated tumor clinically, and a paucity of vascular lesions.

Sumario

Síndrome de Wegener: Caso con Características Extrañas

Preséntase un caso que acusaba las características esenciales del síndrome de Wegener: infección nasal inicial, seguida de fiebre, leucocitosis, eosinofilia, anemia, erupciones cutáneas, artropatía y nefritis terminal.

El estado general del enfermo, que se creyó al principio era septicemia provocada por el *Staphylococcus pyogenes*, continuó sin mayor alteración por la terapéutica, aunque el estafilococo desapareció de las secreciones respiratorias.

En la operación y la autopsia, se encontraron lesiones granulomatosas y vasculares, pareciendo las últimas ser periarteritis nudosa. Una "nariz en silla de montar" fué una complicación extraña, pero típica. De notable interés fueron: un granuloma pulmonar solitario (más bien que lesiones difusas) que simulaba un tumor clínicamente y una escasez de lesiones vasculares.

Resume

Syndrome de Wegener: Un cas avec des caractères inhabituels

Les auteurs rapportent un cas qui présenta les caractères essentiels du syndrome de Wegener: une infection nasale initiale suivie de fièvre, leucocytose, éosinophilie, anémie, éruptions cutanées, arthropaties et néphrite terminale.

L'état général du malade, que l'on pensait d'abord atteint de septicémie à *Staphylococcus aureus* continua à être peu changé par la thérapeutique, bien que le staphyloccoque disparut des secrétions respiratoires.

A l'opération et à l'autopsie, on trouva des lésions vasculaires et granulomateuses, ces dernières ressemblant à celles de la periarteritis nodosa. Un "nez en selle" fut une complication inhabituelle mais caractéristique. D'un intérêt particulier, on trouva un granulome pulmonaire unique (plutôt que des lésions diffuses) qui simula une tumeur cliniquement, et un petit nombre de lésions vasculaires.

Acknowledgments

Drs. J. C. Day, J. N. Wolf, and F. J. Eurs furnished the surgical, roentgenographic, and pathologic data and material from Woman's Hospital, Detroit, which were essential to the elucidation of the above case.

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CHEST CONFERENCE

University of California School of Medicine

CASE UCH 283695

DR. RICHARD E. BRASHEAR[1]: A 47-year-old man was admitted to the hospital on November 20, 1958, because of dyspnea.

History: The patient had felt well until six months previously, when he first noted shortness of breath and a chronic nonproductive cough. Although the symptoms had gradually increased in severity, he was able to continue work as a steam fitter. Three weeks before entry, his dyspnea suddenly became worse, forcing him to curtail his activities. At this time he noticed that bending forward or lying down aggravated the dyspnea.

In the past, the patient had worked as a migrant laborer in Arizona, New Mexico, and the San Joaquin Valley in California. A routine X-ray film of the chest taken in 1954 had shown no abnormalities. His former wife had been treated for pulmonary tuberculosis twenty years ago.

Physical examination: The patient was a well-developed, plethoric man, dyspneic at rest but not in acute distress. His temperature was 37°C., pulse 92, respirations 32, and blood pressure 160/100 mm. Hg. The trachea was shifted to the right. Examination of the left hemithorax showed a striking decrease in lateral expansion, absence of fremitus, hyperresonance to percussion over the upper two-thirds, dullness over the lower one-third, and absence of breath sounds; the right hemithorax was normal. The apical impulse was in the fifth left intercostal space 4 cm. medial to the midclavicular line.

Laboratory findings: The leukocyte count was 8,200 and the packed cell volume 50 per cent. The venous pressure was 7 cm. of saline and the arm-to-tongue circulation time (Decholin) nineteen seconds. The vital capacity was 3.1 liters. An intermediate-strength PPD tuberculin skin test was positive, but coccidioidin (1:100) and histoplasmin skin tests were negative. An X-ray film of the chest (figure 1) showed a left hydropneumothorax with an air-fluid level and a shift of the heart, mediastinum, and trachea to the right.

A thoracentesis on the day of admission yielded 2,680 ml. of air and 1,535 ml. of cloudy yellow fluid. On the following day, two Robinson catheters were inserted into the left pleural space to permit continuous suction. Because of recurrent pneumothorax, intrapleural suction was maintained for sixteen days, during which time the patient received 1 million units of penicillin and 1 gm. of streptomycin daily. His condition was unsatisfactory during this period because of persistent partial collapse of the left lung, recurrent spiking fever, and roentgenographic evidence of fibrothorax formation. No abnormalities were seen during bronchoscopy on December 1. A bronchogram of the left lung at this time, however, showed conglomeration of the bronchi in the lower lobe, but no evidence of intrinsic bronchial disease. Multiple cytologic studies of sputum, bronchial washings, and pleural fluid were negative.

On December 13, the patient underwent decortication of the left visceral and parietal pleura, which was greatly thickened, together with wedge resection of a cavity found in the left upper lobe. The partially collapsed left lung was encased in a thick white fibrous membrane. The cavity, measuring 2 cm. in diameter, was located between the lingula and the anterior segment of the left upper lobe and communicated with both the pleural space and a small bronchus. It was identified as a bronchopleural fistula.

The patient made an uneventful recovery and was discharged two weeks later. Cultures of tissue, pleural fluid, and sputum at the time of dismissal were negative for acid-fast bacilli. The results of diagnostic studies for coccidioidomycosis in December, 1958, and on three subsequent occasions are listed in table 1. A follow-up X-ray film of the chest on August 3, 1959, showed what appeared to be a cavity in the left upper lobe; in a second film in November of the same year (figure 2*A*) the cavity had become much larger and now measured 2 by 3 cm. A third film on January 18, 1960 (figure 2*B*) showed a definite reduction in the size of the cavity. The patient has remained well during this time.

DISCUSSION

DR. JOSEPH J. FURLONG[2]: The most interesting aspects of this case are (*1*) the differential diagnosis, (*2*) the unusual initial complaints, (*3*) the diagnostic and prognostic value of the serologic tests, and (*4*) the postoperative course. From the information in the protocol, I would strongly suspect that this patient had coccidioidomycosis. Our main problem is to decide whether all the features of this case can be related logically to a single diagnosis.

In the diagnosis of coccidioidal infection, a history of exposure in an endemic area or to fomites is essential (1–3). After an incubation period of one to three weeks, symptoms of an influenza-like illness occur in about 40 per cent of patients (4), and X-ray films of the chest may

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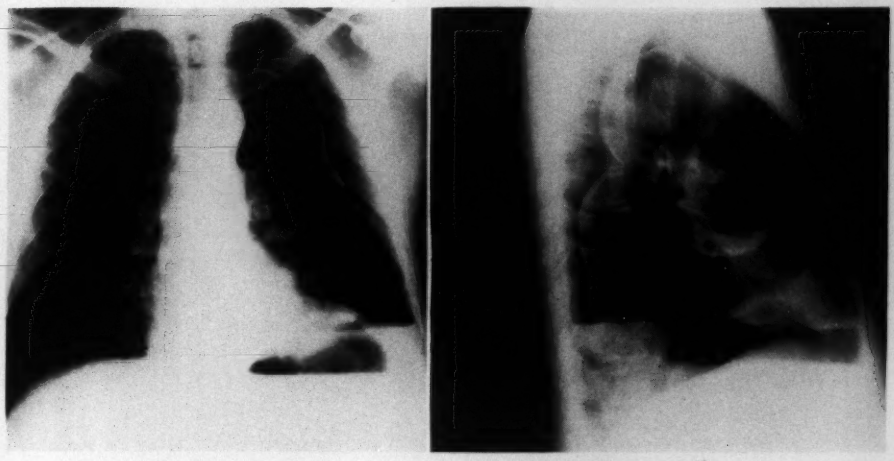

FIG. 1. Roentgenograms of the chest on November 20, 1958, showing left hydropneumothorax.

TABLE 1

RESULTS OF POSTOPERATIVE COMPLEMENT-FIXATION AND PRECIPITIN TESTS

Date Performed	Complement Fixation Test		Precipitin Test
	1:2 Dilution	1:4 Dilution	
12/58	++++	++	Negative
2/59	++++	++++	Negative
3/59	++++	+++	Negative
11/59	Negative	Negative	Negative

reveal bronchopneumonic infiltrates, enlarged hilar lymph nodes, or pleural effusions. At this stage the fungus may be cultured from the sputum, an extremely hazardous procedure for laboratory personnel (5, 6). Cavitation at the site of the original infection occurs in 1 to 3 per cent of primary cases severe enough to require medical attention, or in about 0.1 per cent of all cases of coccidioidomycosis. Typically, these cavities are thin-walled, and there is little or no surrounding inflammatory reaction, in contrast to the usual cavitary lesion of tuberculosis. Of the patients who develop cavities, 58 per cent are asymptomatic. Of the remaining cases, 26 per cent are discovered because of hemoptysis and 16 per cent because of chest pain or nonspecific respiratory symptoms (7).

This patient had a history of repeated exposure in an endemic area. The nonproductive cough and progressive dyspnea during a six-month period probably were caused by primary coccidioidal pneumonia, with or without a pleural effusion. From the operative findings we must assume that he developed a peripheral cavity and that rupture of this cavity into the pleural space three weeks before his admission accounts for the abrupt increase in symptoms, the hydropneumothorax, and the persistent bronchopleural fistula. It should be emphasized that spontaneous hydropneumothorax is a rare complication in coccidioidal infection (8). Smith and associates (4) reported its occurrence in 2.6 per cent and Winn (9) in 2 per cent of patients with cavitary lesions. Combining these figures, it appears that spontaneous hydropneumothorax occurs only once in every 2,500 coccidioidal infections.

Before considering the serologic studies, I would like to comment on the significance of the coccidioidin skin test. Dilutions of 1:10, 1:100, and 1:1,000 are used. The 1:100 dilution is satisfactory for diagnostic screening (10). Dilutions of 1:10 should be reserved for patients who fail to react to the 1:100 dilution, e.g., those who have disseminated coccidioidal infections, and the 1:1,000 dilution for the 2 to 5 per cent of patients (usually women) in whom hypersensitivity is manifested clinically by erythema nodosum (11). The reaction to coccidioidin is of the delayed type. In 60 to 90 per cent of patients the skin test gives a positive reaction ten to forty-five days after exposure and primary infection. The change from a negative to a positive skin test, as in tuberculosis, establishes beyond doubt that infection has occurred (10).

The results of serologic testing (12) in this case provide diagnostic evidence that is unequivocal. In mild infections, precipitins and complement-fixing antibodies are rarely present. In severe

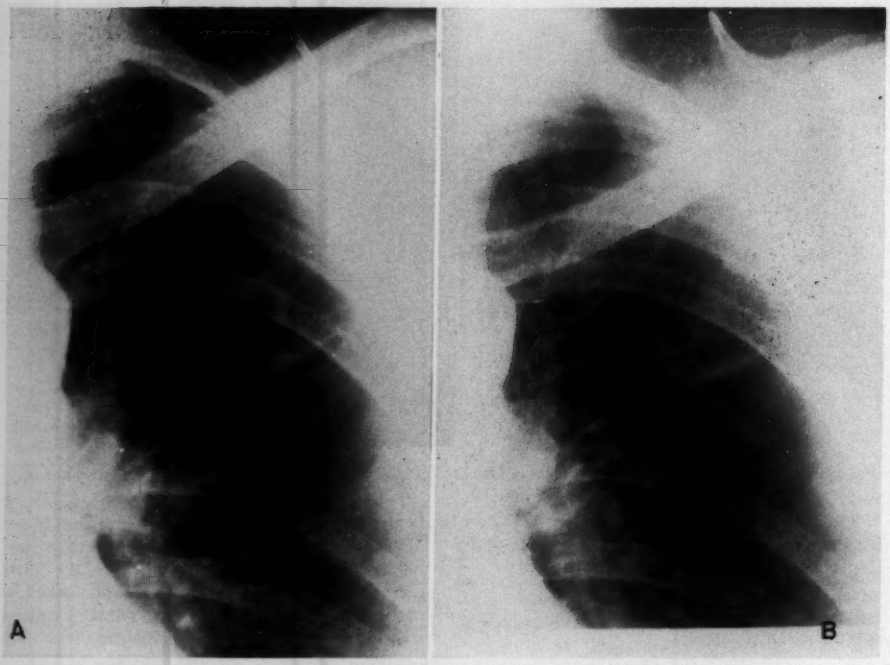

FIG. 2. Follow-up roentgenograms of the chest. (*A*) On November 23, 1459, showing recurrent cavity in the left upper lobe. (*B*) On January 18, 1960, showing decrease in size of the cavity.

primary infections, precipitins appear a few days after the skin test becomes positive, diminish in two to three weeks, and usually disappear in four to five months. They may be absent in disseminated coccidioidomycosis. Complement-fixing antibodies appear somewhat later than precipitins. The complement-fixation titer is a reliable index of the severity of the infection. A rising titer indicates progression, and a falling titer regression of the infection—an important guide to prognosis (13). With progressive dissemination, the titer often becomes extremely high (> 1:16).

The consistently negative precipitin tests in this case support our clinical impression that the infection had occurred at least six months previously. The rise in complement-fixation titer after the operation and its subsequent fall to zero indicate a postoperative exacerbation of the disease, followed by improvement. The rise and fall of the complement-fixation titer show a striking correlation with the development, enlargement, and subsequent decrease in the size of the cavity in the left upper lobe seen roentgenographically.

New cavities frequently develop after surgical treatment (7, 14, 15), an important factor to consider in planning therapy. In a recent report on 77 cavitary cases (7), nonsurgical therapy in 36 cases resulted in closure of the cavities within two years in 50 per cent of the patients so treated, whereas, in 41 cases treated by resection, bronchopleural fistulas subsequently developed in 29 per cent and new cavities in 20 per cent, requiring a total of 8 thoracoplasties and 2 lobectomies. The indications for operation are not the same as in tuberculosis, since complications such as bronchogenic spread, late dissemination, rupture, and secondary infection are rare. Patients with lung cavities are not infectious to others, the cavity wall is thin and the hazards of cutting into infected tissue are great; in addition, the results of nonsurgical treatment are good. Indications for surgical treatment include the presence of a large cavity, more than 4 cm. in diameter, especially when peripherally located, an enlarging cavity, recurrent severe hemoptysis, and a persistent bronchopleural fistula, as in this patient.

Dr. Furlong's diagnoses:

Coccidioidomycosis of lung, with cavitation, left upper lobe
Bronchopleural fistula, due to rupture of cavity, with hydropneumothorax
Fibrothorax

FIG. 3. Section through satellite granuloma, showing spherules (periodic acid-Schiff stain, × 540)

Recurrent cavitation, left upper lobe, following surgery

Anatomical diagnoses:

Coccidioidomycosis of lung, with cavitation, left upper lobe
Bronchopleural fistula, due to rupture of cavity
Fibrous pleuritis

Pathologic Discussion

DR. ROBERT WRIGHT[3]: The surgical specimen consisted of a sheet of thickened pleura, measuring 15 by 10 by 0.5 cm., several small fragments of fibrous tissue, and a 3 by 3 cm. wedge of lung, in the center of which was an irregular, thin-walled cavity. The cavity communicated with a small bronchus and the pleural space, forming a fistula.

Histologically, the pleura was composed of dense fibrous tissue with numerous granulomatous foci containing epithelioid, chronic inflammatory, and multinucleated giant cells. The wall of the cavity and fistula also showed areas of granulomatous inflammation. A satellite granuloma was found in the lung parenchyma peripheral to the cavity wall. Periodic acid-Schiff–stained microsections of the satellite granuloma showed multiple spherules (figure 3). *Coccidioides immitis* was cultured from both the pleural fluid and the tissue removed at operation.

DR. SIDNEY SHIPMAN: I think Dr. Wright's demonstration of the satellite granuloma illustrates one of the problems faced by the surgeon when he resects a coccidioidal cavity. If he attempts to preserve as much lung tissue as possible by doing a segmental or limited wedge resection, small satellite granulomas may be left behind and subsequently lead to recurrent cavitation or bronchopleural fistula formation. The presence of a remaining granuloma undoubtedly was responsible for the recurrent cavitation after operation in this case. Until effective therapy for coccidioidomycosis becomes available, the only way of reducing the high incidence of postoperative complications would appear to be the removal of cavities either by wedge resection with wide margin or by lobectomy as Cotton and Birsner (16) have recently emphasized.

DR. PAUL SLATON: Dr. Furlong, would you comment on the use of amphotericin B? Would you have used it in this case?

DR. FURLONG: As you know, many drugs have been tried unsuccessfully in this disease. Recently, favorable results have been reported following the use of amphotericin B in selected cases (17, 18). It is the treatment of choice in cases of impending or actual dissemination and in meningitis. Other indications include a severe primary infection, a rising complement-fixation titer, evidence of spread to other tissues, and pregnancy. The maximal tolerated dose is 1.0 to 1.6 mg. per kg. of body weight (17), given intravenously in 5 per cent glucose daily or every other day to maintain blood levels of 0.5 to 1.0 γ per ml. (18). Treatment may be continued for three to four months and is monitored by the complement-fixation titer. Amphotericin B also may be given three to four

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weeks preoperatively to prevent the exacerbations which often follow surgical treatment, as in this case. This drug must be used with caution because of its nephrotoxic effects.

Since the diagnosis in this case had not been established prior to surgery, I would not have used amphotericin B.

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May 26, 1960

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Editorial

Long-Term Treatment with Antimicrobials and Anti-inflammatory Steroids in Chronic Bronchitis and Infectious Asthma. Its Potential Value for Rehabilitation and Prevention

The prospect of eradicating tuberculosis in the foreseeable future has stimulated interest in chronic bronchitis, chronic asthma, and their complications, which apparently are on the increase. The socio-economic aspects of these ailments may be more serious in Great Britain than in the United States. However, in this country, too, chronic respiratory disease that causes enormous economic losses and much suffering in the young and the aged is becoming a major concern.

The California Health Survey of 1954–1955[1] disclosed that 6 per cent of days of disability were due to chronic respiratory illnesses, the same percentage as found for arthritis and rheumatism. Asthma and bronchitis were among the ten most frequently disabling medically attended chronic conditions. In 1957, emphysema ranged as the second of ten primary diagnoses occurring most often among male workers for whom disability was allowed under the Social Security Act.[2] According to the U. S. National Health Survey of 1957–1958, the rate of chronic bronchitis in children less than ten years old (about 15 cases per 1,000 population) now is roughly equivalent to the rate among persons fifty-five years of age and older.[3] Since the beginning of the antimicrobial drug era, many thousands of persons with bronchopulmonary diseases, who before would have succumbed to exacerbations, have survived such episodes and grow old with their chronic ailment. Similarly, the life span of patients with tuberculosis has been extended, but many survivors have damaged, scarred lungs. Thus, the number of persons with primary or secondary chronic bronchitis, asthma, bronchiectasis, and emphysema increases steadily, creating a serious problem.

Conventional methods of treatment may relieve distressing symptoms of the chronic pulmonary patient, but apparently they cannot solve the problem of rehabilitation and prevention. Therefore, any novel approach, however unpromising it may appear at first, deserves serious consideration. The chemotherapeutic attack upon the immediate exacerbation, even though it can prevent serious sickness, does not forestall subsequent flare-ups. These relapses account largely for the perpetuation and progression of the underlying disease. For this reason, attempts to control chronic bronchitis and asthma continuously have been based in recent years on the reduction of exacerbations by long-term chemotherapy. Such treatment has proved successful in the management of tuberculosis and rheumatic fever; however, thus far its application in nontuberculous pulmonary diseases has found more opponents than supporters. Since chronic bronchitis and infectious asthma, unlike tuberculosis and rheumatic fever, are not associated with one single, known bacterial pathogen, it is argued from an academic point of view that these respiratory afflictions cannot be controlled by antibacterial prophylaxis. Moreover, such therapy, it is believed, would increase the emergence and spread of treatment-resistant organisms and would be inadvisable also because of possible complications from treatment and the high cost of drugs. Naturally, such an atmosphere of unfavorable opinions has not encouraged extensive research into the problem of chemoprophylaxis in bronchopulmonary diseases. Nevertheless, the results of the limited work done in this field thus far ought to temper the prevalent overskepticism and reduce exaggerated fears to their true proportions.

It is true that the original favorable reports dating back to the beginning of the chemotherapy era were based chiefly on empirical observations.[4–7] However, as early as 1953, McVay and Sprunt,[8] for example, demonstrated statistically that

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chlortetracycline reduced the number of exacerbations in adult patients with bronchiectasis and chronic bronchitis. Grater[9] and others observed similar results in children with asthmatic bronchitis. Among the few recent investigations in this country, the double-blind study by Cherniak and associates[10] of 67 patients with chronic bronchitis, 45 of them with bronchiectasis, confirmed the effectiveness of chemoprophylaxis with tetracycline in significantly reducing lower respiratory illnesses. Their group of patients had been observed from three to eighteen months. On the other hand, in a twelve-week controlled study of 22 patients with diffuse chronic obstructive pulmonary emphysema and a mean maximal breathing capacity of only 35, Hallet and coworkers[11] found no evidence that erythromycin altered the course of the disease.

In Great Britain, where chronic bronchitis has been recognized as a major cause of industrial invalidism, the possible value of long-term chemotherapy in reducing disabling sickness has aroused particular interest. Again, early favorable observations were based chiefly on clinical impressions, but soon were substantiated by large-scale controlled investigations.[12-19] In a double-blind trial by the British Medical Research Council[17] on 122 patients with advanced bronchiectasis, oxytetracycline taken twice a week for one year effected a rapid decrease in sputum volume, a reduction of absenteeism from work, of febrile episodes, and of the number of days confined to bed. Murdoch and associates,[18] in a similar study, observed during two winter seasons a reduction of the number and severity of acute exacerbations in patients with chronic bronchitis who took antimicrobial drugs regularly. The British Tuberculosis Association[19] concluded from its controlled investigations that, among 226 patients with chronic bronchitis, either penicillin V or tetracycline halved the days lost from work, although neither reduced the number of attacks of bronchitis. In the United Kingdom these observations have been accepted as sufficiently encouraging to give long-term chemoprophylaxis a firm place in the treatment of chronic bronchitis.[20]

Fortunately, contrary to all predictions, the incidence of clinically important ill effects from prolonged chemotherapy has been low, especially if parenteral administration and excessive doses of the drugs are avoided. It appears now that minor and major complications from supervised chemoprophylaxis do not occur more frequently than, for example, from desensitization and tonsillectomy, treatments that have been widely accepted for chronic respiratory diseases in spite of their potential hazards. Similarly, an increase of drug-resistant pathogens and clinical superinfections has not emerged as an important problem in ambulatory patients receiving penicillin or other antimicrobials continuously. These observations, recently confirmed also by Dowling and associates,[21] are in conformity with the long experience gained in rheumatic fever prophylaxis. Murdoch and associates[18] even failed to observe a gross disturbance of the normal intestinal flora in patients treated prophylactically with therapeutic doses of tetracycline.

Since the old postulate, "one disease—one agent," is being abandoned in viral respiratory illnesses,[22] it need not be upheld in bacterial bronchopulmonary infections if newer knowledge forces its modification. *Hemophilus influenzae* and pneumococcus may be the most important pathogens in these diseases; quite often, however, they are not predominant or are even absent, and clinically effective chemotherapy is not necessarily paralleled by their eradication.[14, 15] Other observations such as Lees and McNaught's comparative study of sputum from bronchitics and "normal" persons[23] also indicate the limited value of a conservative bacteriologic approach to the diagnosis and treatment of mixed bronchopulmonary infections.

In general, the tetracyclines have been found to be most effective in the treatment of lower respiratory infections, especially for exacerbations. However, penicillin often has proved beneficial, too,[14, 19] and, if penicillin G is used to the greatest possible extent, the cost of long-term treatment is substantially reduced. Still, the economic gains of

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chemoprophylaxis, if measured against its cost, often may be slight[19]; but, from a medical point of view, improvement not obtainable from other treatments would justify even additional expense. Moreover, most investigations have been conducted on patients with advanced disease complicated by irreversible lesions; therefore they cannot demonstrate the full potential value of any treatment.

Conceivably, antimicrobial therapy achieves better results in uncomplicated chronic bronchitis than in chronic asthma and emphysema, in which transient bronchial obstruction or permanent pulmonary damage often outweigh the infection in importance. Anti-inflammatory hormones now can control bronchial obstruction more completely than expectorants and bronchodilators alone, and the value of short-term steroid treatment for tiding patients with severe asthma over critical periods has been generally recognized. However the prolonged use of these hormones for continuous control of this disease still is a controversial subject. Nevertheless, an increasing number of writers agree that, in spite of certain risks involved, a prolonged regimen with anti-inflammatory steroids makes it possible to keep many asthmatic patients well who without such treatment would have become invalids or would have died.[24-29] Combined antimicrobial-steroid therapy, which now is advocated by some writers in the treatment of tuberculosis even as a routine measure,[30] seems rational also in other respiratory diseases in which infection and inflammation coexist.

Judging from the development of chemotherapy in tuberculosis, extended management of this type gradually will be accepted also in chronic bronchitis and asthma, as favorable reports continue and as safer, more potent drugs are developed. From the viewpoint of preventive medicine, it seems particularly important to explore further the value of chemoprophylaxis, not only in advanced cases, but also in earlier ones when irreversible pulmonary damage still may be prevented and cure achieved. Obviously, in chronic bronchopulmonary diseases with their many variables, long-term, "double-blind" therapeutic trials would leave the urgent problem of possible prevention unsettled for many years. Therefore instead of waiting for absolute statistical proof, knowledge obtainable by other methods may be sufficient for at least tentative conclusions.[31]

This writer has given long-term treatment with antimicrobial agents and steroids in chronic bronchitis and infectious asthma since these drugs became available. In his experience, the therapeutic results may have been negligible in some elderly persons with advanced emphysema, but in young patients with reversible pathology the improvement achieved often approached cure.[32-36] Thus, the reduction of disabling sickness, a major goal of rehabilitation in bronchopulmonary diseases, became most obvious in children receiving long-term chemoprophylaxis, in conjunction with steroids, if necessary. Absenteeism among these children remained consistently at the over-all level, in contrast to the excessive loss of school days observed in children with chronic bronchitis and asthma who were treated with conventional methods.[33-36] The protective value of chemoprophylaxis was evident also during times of increased respiratory illnesses in the community, including the epidemic of Asian influenza which proved particularly serious for the vulnerable.[37, 38] Of 96 children and adults with chronic bronchitis or asthma who had an influenza-like illness between September, 1957, and March, 1958, and received antimicrobial drugs as primary treatment, 86 recovered completely within one week. Only one patient who suffered from extensive bronchiectasis developed pneumonia and required hospitalization. During that critical seven-month period in 1957 and 1958, absences from school totaling more than four weeks occurred in only 3 per cent of children with chronic bronchitis or asthma who received chemoprophylaxis, against 26 per cent of other children with these ailments who had not been given such treatment consistently.

The capacity of prolonged chemotherapy to decrease major respiratory flare-ups has continued through the years. In a group of 214 patients with

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chronic bronchitis or asthma, the rate of pneumonia attacks and hospitalizations for respiratory illnesses was 17.4 per 100 patient-years before they started chemoprophylaxis. That rate dropped to 5.4 per 100 patient-years of exposure to chemoprophylaxis. All of these 214 patients, 118 less than sixteen years old, received such treatment for at least one year. Eighty-nine received chemoprophylaxis, mostly penicillin and/or tetracyclines, continuously or intermittently for five to eleven years. Thirty-six patients were given steroids for three to eight years.

The reduction of additional severe pulmonary insults influenced favorably the respiratory and general health of the patients, especially if the disease was not too far advanced. The halt in further progression of the ailment, the subsidence of distressing symptoms, and the increase in working capacity produced a new feeling of well-being, even if vital capacity studies and roentgenograms failed to show an "objective" improvement. However, in cases of early chronic bronchitis and asthma, there was tangible evidence that the course of a progressing disease had been changed manifestly. The regression of reversible pathology, including mild bronchial dilation, could be observed roentgenographically,[39] and the vital capacity often reached normal values.[35, 36] Among 30 patients with chronic asthma who were between the ages of ten and twenty-one years (11 of them more than sixteen) the total and first-second vital capacities were normal in only 7 when they were first examined. During long-term antimicrobial therapy, in many cases with concomitant steroids, these values gradually became normal in 11 additional cases. In another group of 30 patients of a similar age distribution but who had started treatment when younger than ten, the total and first-second vital capacities were normal in 25. Most gratifying was the fact that 14 of these 30 children and young adults had been without chemoprophylaxis for one to six years at the time of the follow-up examination.

Whether or not an actual "cure" had been achieved in any case, in many of the 214 patients observed between 1948 and 1958, a chronic ailment apparently had ceased to exist. At any rate, in the average case, the benefit from prolonged chemoprophylaxis outweighed by far its potential hazards. During a total of 917 patient-years of exposure to treatment, none of the patients, including those taking steroids in association with antimicrobial drugs, had an overwhelming infection and no overgrowth of *Staphylococcus aureus* was noticed in the sputum or the saliva.

Valuable as long-term chemoprophylaxis can be in chronic bronchitis and infectious asthma, the prevention of these diseases would make such involved treatment unnecessary. Adequate and sufficiently long antimicrobial treatment of streptococcal throat infections can spare many patients a prolonged prophylactic regimen with these drugs for rheumatic fever. Similarly, prompt and complete eradication of pulmonary infection and bronchial obstruction—but even more the prevention of such insults—may well avert potentially chronic lung damage.

The present writer firmly believes from his daily experience that early antimicrobial therapy, with or without anti-inflammatory steroids, can prevent or minimize significant bronchopulmonary illnesses in previously healthy persons, as it can in chronic pulmonary patients. This is in contrast to incessant claims by others that chemoprophylaxis is without value in reducing severe sickness and complications from common viral respiratory infections. Actually, thus far no well-controlled studies have demonstrated that chemoprophylaxis, using all available agents, failed to prevent bacterial pneumonia as a complication of influenza. Although some publications may suggest that the risk of complications from measles is greater when antimicrobial drugs are given prophylactically,[40] others indicate that this risk is smaller.[41, 42] Many writers believe that "nonspecific" respiratory infections are not aborted by antimicrobial agents, but Ritchie,[43] among others, found that such treatment, by suppressing the bacterial component, reduced disabling sickness even in the common cold.

Surely, further research in this important field is needed badly. A fresh, bold approach to chemoprophylaxis not only may solve the problem of controlling chronic pulmonary diseases, but also may contribute to the prevention of these ailments by curbing acute, severe respiratory illnesses.

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Notes

CHANGES IN THE LENGTH OF MYCOBACTERIUM LEPRAE MURIUM IN THE EVOLUTION OF MURINE LEPROSY IN MICE

In the studies of evolution of murine leprosy in mice, the present writer[1] reported that elongation of bacilli was observed in the first six to seven weeks following infection and that short bacilli became dominant thereafter. Quantitative data on the length of bacilli were not given at that time.

Recently, limited multiplication of *Mycobacterium leprae murium* in tissue culture has been reported by several groups of investigators.[2-5] No statement on the changes of bacillary length was made. The initial bacilli were obtained from animals three weeks following infection in one group and eight to twelve weeks in another. It is difficult to say whether the limited increase in the number of bacilli was caused by splitting of long forms which might be present in the initial bacilli of the cultures. In order to clarify this point, a quantitative study on the changes in the length of bacilli in the evolution of murine leprosy seems to be desirable.

The materials originally used in evolution studies were re-examined and the length of bacilli was estimated in the mesentery, spleen, and liver. In addition, data obtained from omental lesions as used in other experiments were included. The results are reported here.

Mesenteric spreads and smears of spleen and liver of mice prepared at various intervals following infection with *Mycobacterium leprae murium* were used for this study. The methods of study are reported elsewhere.[1] Briefly, mice were inoculated intraperitoneally with 0.5 ml. of a 1:30 suspension made from omenta and pelvic fatty pads from mice infected four to five months previously. Animals were sacrificed at one, three, five, seven, eleven, and fourteen days, and thereafter at weekly intervals for ten to thirteen weeks. Ten animals were used for each preparation except for spleen and liver at the end of three months, for which 20 animals were employed for each suspension. Suspensions of spleen and liver were made from pooled organs by homogenizing the tissue in a Waring Blendor for three minutes (six minutes for tissues at the end of three months). Smears were made from the original or diluted suspensions.

Mesenteric spreads were prepared by placing a loop of intestine on glass slides. After drying, the intestine and blood vessels were cut off.

Smears were also made from omental lesions used in the cultivation of *M. leprae murium* in tissue culture.[6] Omental lesions were obtained from mice at various stages following infection, ranging from 2 weeks to 180 days. Suspensions (1:100) were made by grinding the omental masses with fine sand in a mortar. The bacilli were stained with a slightly modified Ziehl-Neelsen technique.[1]

The length of bacilli was estimated by inserting a scale in the eyepiece, each division being equivalent to 0.55 μ. The bacilli were classified as follows:

Class I: Less than 4 divisions
Class II: 4 and 5 divisions
Class III: 6 and 7 divisions
Class IV: 8, 9, and 10 divisions or more

The percentage of each class was conveniently recorded by using a blood cell calculator. The weighted average length of bacilli from mesentery, spleen, liver, and omentum, respectively, was estimated as follows:

$$\frac{\text{Per Cent in Class I} \times 2 + \text{Per Cent in Class II} \times 4.5 + \text{Per Cent in Class III} \times 6.5 + \text{Per Cent in Class IV} \times 9}{100} \times 0.55 = \text{Average length of bacilli in } \mu$$

Mesentery: Elongation of bacilli was observed in the mesentery as early as the fourth day of the infection. The length increased steadily, reaching a maximum of about 2.6 times the initial size on the eleventh day. It remained more or less constant until about the end of the sixth week, when a decrease was noticed. By the tenth week, when observations were concluded because of heaviness of the lesions, short bacilli had become dominant but the average length was still longer than at the beginning (figure 1).

Spleen and liver: Changes in the length of bacilli in the spleen and liver showed a pattern similar to that observed in the mesentery, except that the

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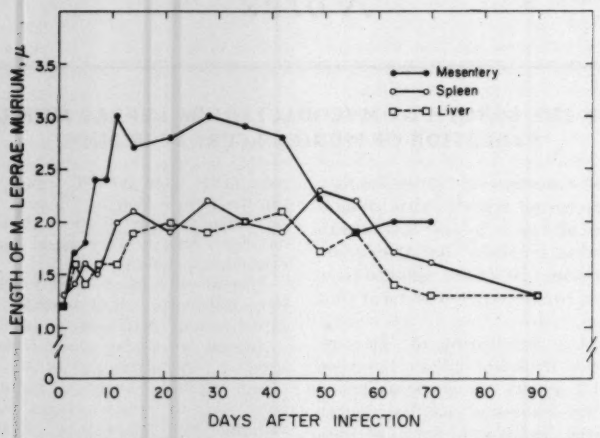

FIG. 1. Changes of the length of *M. leprae murium* in mesentery, spleen, and liver of mice. Each point represents the average length of bacilli as estimated from a total of 500 bacilli, except those for the mesentery in which each point represents 5,000 bacilli.

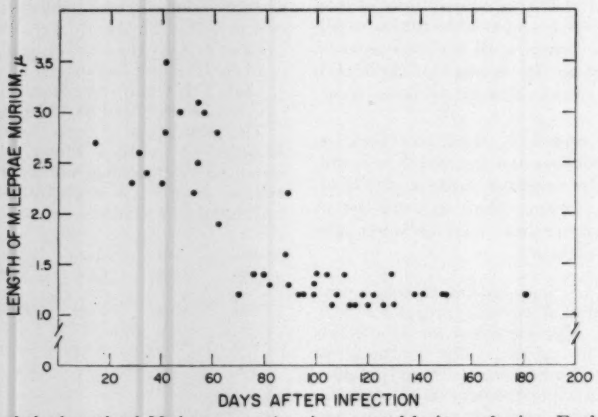

FIG. 2. Changes of the length of *M. leprae murium* in omental lesions of mice. Each point represents the average length as estimated from a total of 500 bacilli.

bacilli were shorter throughout the infection. The maximal length of bacilli was about 1.8 times the initial size. At the end of three months, all bacilli were in short forms (figure 1).

Omental lesions: Estimation of the length of bacilli in omental lesions was made in 43 animals sacrificed from 2 weeks to 180 days following infection. Longer bacilli of 2.2 to 3.5 μ were observed between the second and ninth weeks of infection. In about the tenth week, the long forms decreased rather abruptly, and short ones were dominant thereafter. From approximately the one hundred and tenth day, very short bacilli with an average of 1.1 to 1.2 μ were observed throughout the remainder of the experiment. The average length of bacilli at various intervals is shown in figure 2.

These animals were infected with inocula from mice that had been infected four to five months previously. As stated above, the length of bacilli in the inocula was 1.1 to 1.2 μ. Calculated from these figures, the maximal increase in bacillary length in omental lesions was similar to that in the mesentery, i.e., about 2.6 times the initial size.

The length of *M. leprae murium* showed a maximal increase of 2.6 times the initial size in the mesentery and omental lesions, but only a 1.8-

fold increase occurred in the spleen and liver. The homogenizing process used in the preparation of homogenates of spleen and liver may account for these differences. During the three-minute homogenization in the Waring Blendor, it is possible that some bacilli, especially those which were ready to divide, split into short ones.

On the other hand, the omental suspensions were prepared by gentle grinding of the lesions with sand. It seems that this process does not appreciably change the bacillary length, as a similar increase was observed in the mesentery, which was not treated by any mechanical means.

The method for estimation of the length of bacilli as used in the present study does not give the absolute length of the organisms. The estimated values are useful for the comparative studies of bacillary length in various stages of the infection and in different tissues. The four classes of lengths are convenient for measuring a number of bacilli in a short time. Individual bacilli vary in length from 1 to more than 10 divisions of the scale, i.e., from about one-half to 5.5 μ. When the long forms are dominant, the majority of bacilli fall into classes II and III. Since the range of these two classes is narrow, there will be little difference between the estimated and the absolute length of the bacilli. Greater variations in length occur when the short forms are dominant. In the later stage of the infection, the majority of bacilli measure only one to two divisions, with an average length of less than two in class I. In this condition the estimated value deviates slightly from the absolute length, e.g., 1.1 μ for the former and 0.9 μ for the latter. Therefore, the actual maximal increase in the length of bacilli in the present study was probably more than 2.6 times.

Elongation was also observed in *M. leprae murium* grown in cultures of macrophages, in systems in which marked increases in numbers occurred.[6] Elongation was observed on the fifth day of cultivation and maximal length was usually reached at two to three weeks, a phenomenon similar to that found *in vivo* in the present study.

Recently, limited multiplication of *M. leprae murium* in tissue culture has been reported by several groups of investigators.[2-5] The numbers of bacilli increased 2- to 3-fold and in one instance to 5-fold in a period of three to six weeks. No statement on the changes of bacillary length was made. The initial bacilli were obtained from animals three weeks following the infection in one group and eight to twelve weeks in another. Since long forms are dominant from two to nine weeks following the infection, it seems likely that long organisms will split into small ones in systems which, at the best, are not highly conducive to bacterial growth. Without control studies, taking into account bacillary length, an increase of 2- to 3-fold in numbers of bacilli, caused by splitting of long forms, might be attributed to successful cultivation. Therefore, in cultivation studies of *M. leprae murium* attention should be directed, not only to increases in numbers, but also to changes in bacillary length.

In summary: Changes in the length of *Mycobacterium leprae murium* in the evolution of murine leprosy were studied in mice. Elongation was observed as early as the fourth day of the infection, reached a maximum on the eleventh day and remained at maximal length until the end of six to nine weeks when the length began to decrease. Short bacilli became dominant thereafter, reaching a minimal size 110 days after infection.

The maximal increase was at least 2.6 times the initial length in bacilli observed in lesions of the mesentery and omentum, and 1.8 times the initial length in those seen in the spleen and liver.

Changes in bacillary length are discussed in relation to interpretation of the results of studies of growth of *M. leprae murium* in tissue culture.

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THE EFFECT OF PYRIDOXINE ON ISONIAZID SERUM CONCENTRATIONS IN MAN[1]

The oral administration of pyridoxine effectively protects humans against the peripheral neuritis which may follow the administration of isoniazid,[2] especially when isoniazid is given in high doses. *In vivo* experiments in mice have indicated that very large doses of pyridoxine may cause some inhibition of the antituberculous effect of isoniazid.[3] Other animal experiments[4] and some experience *in vitro* [5, 6] had previously failed to show this inhibitory effect, but other *in vitro* studies[7] had indicated the opposite. In consequence of the questions raised by these observations and because of the importance of obtaining a maximal antituberculous effect with isoniazid in humans with tuberculosis, an experiment was planned to test the effect of pyridoxine on serum concentrations of antimycobacterially active (presumably "free" isoniazid) substances.

Seventeen tuberculous patients at Colorado General Hospital were studied on the following protocol:

All medications except streptomycin were discontinued for forty-eight hours. Each "test dose" of isoniazid was 4 mg. per kg. to the nearest 25 mg.

First day—the test dose of isoniazid was given three times daily.

Second day—same as first day.

Third day—the test dose of isoniazid was given at 7 a.m. followed by a fat-free breakfast at 8.30 a.m. A blood sample was drawn at 1.00 p.m. At 1.30 p.m. the patients were given the test dose of isoniazid plus 200 mg. of pyridoxine. Again at 5.00 p.m., another test dose of isoniazid was given.

Fourth day—the test dose of isoniazid was given three times daily plus 200 mg. of pyridoxine with morning medication.

Fifth day—the test dose of isoniazid plus 200 mg. of pyridoxine was given at 7.00 a.m. followed by a fat-free breakfast at 8.30 p.m. A blood sample was drawn at 1.00 p.m. Regular medications were resumed at this time.

After the blood samples were drawn, they were allowed to clot, were centrifuged, and the serum was pipetted into sterile tubes and frozen until the time of the assay. All of the sera were assayed on the same day by a bioassay technique.

TABLE 1

SERUM CONCENTRATIONS OF ANTIMYCOBACTERIALLY ACTIVE ("FREE ISONIAZID") SUBSTANCES IN γ PER ML. SIX HOURS AFTER TEST DOSES OF ISONIAZID AND ISONIAZID PLUS PYRIDOXINE

Isoniazid Inactivation Status	Patient's Initials	Test Doses	
		Isoniazid	Isoniazid Pyridoxine
"Rapid"	V.S.	<0.2	<0.2
	A.E.	<0.2	<0.2
	A.A.	<0.2	<0.2
	F.O.	<0.2	<0.2
	H.Ra.	<0.2	<0.2
	G.O.	0.2	0.2
	H.Ro.	0.2	0.2
	J.A.	0.2	0.2
	G.S.	0.2	0.2
"Intermediate"	H.B.	0.4	0.4
"Slow"	S.L.	0.8	0.8
	A.F.	0.8	0.8
	P.H.	1.6	1.6
	J.G.	1.6	1.6
	M.M.	1.6	1.6
	P.R.	1.6	1.6
	F.R.	3.2	3.2

The observations made in the 17 patients are shown in table 1. The patients are listed in ascending order by the concentration of "free isoniazid" found six hours after the test was done of isoniazid alone.

The findings after the addition of pyridoxine were the same in all patients with one exception: *H. Ra.* showed <0.2 γ per ml. after isoniazid alone and 0.2 γ per ml. (or a one-tube higher concentration) with pyridoxine added.

The bioassay for "free isoniazid" roughly measures the concentration of substances having

[1] This study was supported by a grant from the Medical Research Committee of the American Trudeau Society, medical section of the National Tuberculosis Association; and by a contract (#SAph 70474) with the U. S. Public Health Service.

[2] BIEHL, J. P., AND VILTER, R. W.: Proc Soc Exp Biol Med, 1954, *85*, 389.

[3] MCCUNE, R., *et al.*: Amer Rev Tuberc, 1957, *76*, 1100.

[4] GRUNBERG, E., AND BLENCOWE, W.: Amer Rev Tuberc, 1955, *71*, 898.

[5] BRUN, J., *et al.*: C R Soc Biol, 1954, *148*, 1817.

[6] UNGAR, J., *et al.*: Lancet, 1954, *2*, 220.

[7] BOONE, I. U., *et al.*: Amer Rev Tuberc, 1957, *76*, 568.

an isoniazid-like inhibitory activity against *M. tuberculosis*.[8-10]

A more precise meaning of the concentrations reported is as follows:

Test Reading γ *per ml.*	*Serum-"free Isoniazid" Range* γ *per ml.*
<0.2	0–0.1
0.2	0.2–0.3
0.4	0.4–0.7
0.8	0.8–1.5
1.6	1.6–3.1
3.2	3.2–6.3

An occasional one-tube variation has been regarded as an inherent error of the method. The findings after the two different test doses in this study are therefore convincingly equivalent.

In another study to be published, similar results were observed in 9 patients.[11]

In summary: Serum concentrations of antimycobacterially active "free-isoniazid" substances after the oral administration of isoniazid to humans are not altered by the concurrent administration of pyridoxine.

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[8] Bell, J. C., and Riemensnider, D. K.: Amer Rev Tuberc, 1957, *75*, 995.

[9] Morse, W. C., *et al.*: Trans 15th Conf on Chemother Tuberc, V. A., Army & Navy, 1956, p. 283.

[10] Mandel, W., *et al.*: Proc Soc Exp Biol Med, 1956, *91*, 409.

[11] Levy, D., *et al.*: Amer Rev Resp Dis, 1960, *81*, 102.

BIOLOGIC PROPERTIES OF MYCOBACTERIA ISOLATED FROM DOGS

Various strains of mycobacteria have been isolated by the present writers[1, 2] from the lymph nodes of ownerless dogs captured in Fukuoka Prefecture, Japan. The phage-susceptibility pattern of these mycobacteria has been reported elsewhere.[3] The purpose of the present paper is to report their drug-susceptibility and niacin-producing ability.

Thirteen strains of mycobacteria isolated from dogs were examined for: their resistance to streptomycin, PAS, isoniazid, neotetrazolium, and potassium tellurite; their niacin-producing ability; and their virulence for the chicken.

For the susceptibility test against antituberculous drugs, egg medium containing different concentrations of each drug was used; against neotetrazolium and potassium tellurite, the Dubos liquid medium was used. The amount of inoculum was 0.1 mg. in each case. Niacin formation was tested on egg medium according to Runyon's method.[4] Five milligrams of each organism were inoculated subcutaneously into white leghorn chickens. After three months, these chickens were autopsied and examined macroscopically and microscopically. Cultures for the bacilli were made from the spleen.

As may be seen in tables 1 and 2, these mycobacteria could be divided into two groups (*A* and *B*) according to their properties. All seven strains of *Group A* were, with rare exception, highly susceptible to all of the drugs used, and gave positive reactions to niacin tests. These results, together with the phage-susceptibility pattern and the pathogenicity for guinea pigs reported in the previous paper,[3] indicate that these strains correspond to human tubercle bacilli.

However, the strains of *Group B* were resistant to one or more of the drugs used, and gave negative niacin test results. These properties appeared similar to those of the avian mycobacteria, but these strains proved to be avirulent for chickens. The results indicate that these strains resembled the unclassified mycobacteria, especially the nonchromogens.

In summary: The present results confirm the conclusion reached in the previous report[3] that seven strains of mycobacteria isolated from dogs (*Group A*) resembled human tubercle bacilli, while some of the *Group B* strains could possibly be classified among the unclassified mycobacteria.

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March 8, 1960

[1] Toda, T., *et al.*: Tokyo Med J, 1957, *74*, 723.
[2] Toda, T., *et al.*: Studies on mycobacteria isolated from dogs (in preparation).
[3] Takeya, K., *et al.*: Amer Rev Resp Dis, 1960, *81*, 674.
[4] Runyon, E. H., *et al.*: Amer Rev Tuberc, 1959, *79*, 663.

TABLE 1

SUSCEPTIBILITY TO ANTITUBERCULOUS DRUGS OF MYCOBACTERIA ISOLATED FROM DOGS

Group	Strain	Streptomycin γ per Ml.				PAS γ per Ml.				Isoniazid γ per Ml.			
		0	10	100	1,000	10	100	1,000	10,000	50	100	200	400
A	Nogata 23	+++	++	−	−	−	−	−	−	−	−	−	−
	Nogata 70	+++	−	−	−	−	−	−	−	−	−	−	−
	Kasuya 13	+++	−	−	−	−	−	−	−	−	−	−	−
	Kasuya 23	+++	−	−	−	−	−	−	−	−	−	−	−
	Mizuma 21–1	+++	−	−	−	+	−	−	−	−	−	−	−
	Fukuoka 8	+++	−	−	−	++	−	−	−	−	−	−	−
	Fukuoka 14	+++	−	−	−	−	−	−	−	−	−	−	−
B	Kasuya 24	+++	−	−	−	++	+	+	−	−	−	−	−
	Kasuya 27	+++	+++	+	−	+++	+++	+++	+	+++	+++	+++	+++
	Ukiha 1–1	+++	++	−	−	+++	+++	+++	−	−	−	−	−
	Ukiha 1–2	+++	++	+	−	+++	+++	+++	−	−	−	−	−
	Yakatabaru 3	+++	−	−	−	+++	+++	++	−	−	−	−	−
	Zieitai 97	+++	−	−	−	++	++	++	−	−	−	−	−
Human	H37Rv	+++	−	−	−	−	−	−	−	−	−	−	−

TABLE 2

SEVERAL BIOLOGIC PROPERTIES OF MYCOBACTERIA ISOLATED FROM DOGS

Group	Strain	Neotetrazolium γ per Ml.					Potassium Tellurite γ per Ml.				Niacin Test	Phage Susceptibility[2]	Pathogenicity for the Guinea Pig [1–3]
		0	3	10	30	100	0	10	100	1,000			
A	Noogata 23	++	−	−	−	−	++	−	−	−	++	+++	++
	Noogata 70	+++	+++	++	+	+	+++	+++	+++	−	++	+++	++
	Kasuya 13	+++	−	−	−	−	+++	++	+	−	++	++	++
	Kasuya 23	+++	+++	++	−	−	+++	++	+	+	++	+++	++
	Mizuma 21–1	+++	−	−	−	−	+	−	−	−	+++	+++	++
	Fukuoka 8	+++	+++	++	−	−	+++	+++	++	−	+++	+++	/
	Fukuoka 14	+++	++	+	−	−	++	+	+	+	+++	/	/
B	Kasuya 24	+++	+++	+++	+++	+++	+++	+++	+	+	−	−	−~±
	Kasuya 27	+++	+++	+++	+	+	+++	+++	+++	+++	−	−	−~±
	Ukiha 1–1	+++	+++	+++	+++	+++	++	+	+	+	−	−	−~±
	Ukiha 1–2	+++	+++	+++	+++	+++	+	+	+	+	−	−	−~±
	Yakatabaru 3	+++	+++	+++	+++	+++	++	++	+	+	−	−	/
	Zieitai 97	+++	+++	+++	+++	+++	+++	+++	++	+	−	−	−~±
Human	H37Rv	+++	−	−	−	−	+++	++	+	−	+++	+++	+++

Legends to Tables 1 and 2

	Drug-susceptibility Tests	Niacin Test	Phage Susceptibility	Pathogenicity for the Guinea Pig
+++ =	Full growth	Strongly positive	Complete lysis	
++ =	Moderate growth	Moderately positive	Lysis with resistant colonies	Virulent
+ =	Weak growth	Weakly positive		
− =	No growth	Negative	No lysis	
−~± =				Avirulent or weakly virulent
/ =			Not examined	Not examined

Correspondence

STUDIES ON THE PH OF THE RESPIRATORY TRACT

To the Editor of the American Review of Respiratory Diseases:

In a previous communication (Jacobs, S., and Papperman, S. M.: J Pediat, 1953, *53*, 298), it was suggested that the pH of secretions of the respiratory tract might reflect basic tissue states of health or disease. The pH of endotracheal aspirate in the healthy newborn was shown to average 7.60 at birth and 7.45 at the end of twenty-four hours of extra-uterine life; this drop in pH apparently bore no relationship to the organism isolated from these secretions, the latter being chiefly *P. aeruginosa*, *α-streptococcus*, *E. coli*, *N. flavescens*, *B. subtilis*, diphtheroids, and *D. pneumoniae* (rough).

In extending these observations to adults, we had planned to study at least 100 persons bronchoscoped for a variety of clinical indications, assuming that among them we would find a sufficient

TABLE 1

pH AT VARIOUS LEVELS

Case	Diagnosis	Sputum	Trachea	Main Bronchus		Organisms Isolated from Secretions
				Right	Left	
J.M.	Bronchogenic cyst	9.6	10.0	8.2	9.2	Nonhemolytic streptococci, *Staph. citreus*, diphtheroids
R.D.	Undiagnosed	8.5	8.5	7.5	7.8	*N. flavescens*, *α* - hemolytic streptococci
L.M.	Hemoptysis, cause undetermined	7.0	6.6	8.0	8.5	Nonhemolytic streptococci, *Staph citreus*, *N. flavescens*
O.F.	Bronchiectasis	6.9	6.0	6.5	5.9	Nonhemolytic streptococci, diphtheroids
G.C.	Suppurative bronchitis	6.8	6.2	5.0	5.2	*Staph. albus*
K.S.	Pneumonia, left upper lobe	7.0	6.4	5.9	6.0	Nonhemolytic streptococci, *Staph. albus*, neisseria
W.B.	Chronic bronchitis	6.8	6.4	4.0	4.2	No growth
J.K.	Bronchiectasis	6.9	6.5	5.0	4.8	Nonhemolytic streptococci, *A. aerogenes*, *B. proteus*, *N. catarrhalis*, *Staph. albus; D. pneumoniae*, *N. flavescens*
T.P.	Abscess, left lung	7.3	6.8	6.0	5.8	*Proteus vulgaris; Staph. albus*, nonhemolytic streptococci
C.P.	Abscess, right upper lobe	7.3	6.9	6.0	6.7	No growth
M.O'B.	Pneumonia right lung, unresolved	6.6	5.7	6.2	5.6	*Staph. albus*, hemolytic streptococci, *N. catarrhalis*
F.K.	Carcinoma, right lung	6.8	6.0	5.2	5.6	*Staph. aureus*, *B. hemolyticus* streptococci
G.S.	Carcinoma, right lung	7.6	6.9	6.4	6.9	*Staph. albus*, *D. pneumoniae*, *α* - hemolytic streptococci, *N. catarrhalis*
R.P.	Carcinoma, right lung	6.9	5.2	4.6	4.2	*Staph. aureus*, *Proteus vulgaris*, pseudomonas
J.L.	Carcinoma, right lung	7.0	7.0	6.4	6.0	Nonhemolytic streptococci, *Staph. albus*, *B. hemolyticus* streptococci
L.T.	Carcinoma, right lung	7.2	7.9	7.3	7.0	*Staph. albus*

number of "normals" to provide data of clinical usefulness. Unfortunately, we had studied only 16 patients when we were obliged to discontinue the project because the special laboratory was being demolished in the course of a hospital rebuilding program. It will be at least another year before this project can be resumed; since we have been unable to find any similar data elsewhere recorded, we believe that even these fragmentary results justify reporting.

In general, the technique of Steinman (Pract Otorhinolaryng, 1953, *15*, 329), the only previous worker in this field, was used but attempts were made to learn whether changes in acid-base relationships are due to alterations of bacterial ecology. Bronchoscopy was performed in the usual manner, secretions were obtained, cultures were prepared, and measurements of pH made on the intact respiratory mucous membranes. After experimentation, a flexible gastric electrode was found to be satisfactory for our purpose. The electrode was passed through the bronchoscope until the tip encountered the mucous membrane of the trachea, the right main bronchus, and the left main bronchus, respectively. At each of these points a direct reading was taken using the Beckman Model G potentiometer. Care was exercised not to traumatize the mucosa or to produce bleeding, either of which accidents would have invalidated the observations by the presence of blood or tissue fluids. An aliquot of secretions was tested for pH as soon as obtained, and the remainder was cultured on the following media: blood agar, Levine eosin-methylene blue agar, nutrient agar, antibiotic number 2 media, thioglycollate broth, thiol media, and Sabouraud's medium.

Observations on 16 patients are presented in table 1. Three patients without abnormality of the tracheobronchial mucosa or intrathoracic disease were found to have pH readings in the trachea of 6.6 to 10.0; that of the right main bronchus from 7.5 to 8.2, and of the left main bronchus from 8.5 to 9.2. By way of contrast, in 8 patients with either bronchoscopic evidence of endobronchial disease or with other indications of bronchopulmonary suppurative disease, the tracheal values averaged 6.36, the right main bronchus 5.57, and the left main bronchus 5.53. Among 6 patients with bronchogenic carcinoma (affecting chiefly the right lung) the corresponding values were 6.6, 5.98, and 5.94. It may be noted that there was no striking difference among these groups in the organisms isolated from the respiratory tract secretions. Accordingly, there is no reason to believe that the differences in pH are due solely to the organism growing therein; possibly the differences may reflect some basic tissue phenomenon either engendered by or permitting the occurrence of specific morbid states.

It is interesting that in subjects without manifest abnormality of the tracheobronchial tree, the sputum was alkaline, its pH paralleling that of the tracheal wall; in bronchitis or when there was carcinoma, the sputum was neutral or slightly acid without relation to the pH of the tracheal wall. Apparently in health as well as in disease, the pH of the tracheobronchial tree reflects a gradient: the mucosa of the trachea is alkaline or slightly acid, that of the peripheral airways becoming progressively acidic in caudal direction. The "ordinary inhabitants" of the air passages seem not to be responsible for this gradient effect. Whether microorganisms capable of producing specific morbid states may in themselves affect the pH of the walls of the respiratory tract or of the sputum is not known.

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[1] The writers are grateful to Dr. Sam A. Threefoot, Director of Research of Touro Infirmary, for his helpful suggestions during the course of the project and in preparation of the manuscript.

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THE PROPHYLAXIS OF TUBERCULOSIS: FACTS VERSUS DREAMS

To the Editor of the American Review of Respiratory Diseases:

In the wake of the Arden House Conference, there are inevitable questions in the minds of many workers as to changes of program which should be made now. Interpretations are proceeding, and policies are being reviewed across the country to determine their suitability in this new era of attempted "tuberculosis eradication."

This writer takes no serious exception to any part of the Arden House report. It is stimulating and constructive. A new feature in the report is the suggestion that isoniazid prophylaxis may come to play a large part in the "eradication" campaign.

The report[1] makes no specific recommendation for a program of prophylaxis at the present time. It recognizes the need for further study. To quote:

> (There is) recognition of the importance and potential significance of the current isoniazid prophylaxis field study of the Tuberculosis Program of the U. S. Public Health Service. . .
>
> It is too soon to draw any conclusions from this study. Will isoniazid prevent infection? Will it prevent active disease in tuberculin-positive adults? It seems likely that it is effective while given. How long the prophylactic effect will last after drug-taking has stopped—which is the crux of the research—remains to be seen.

Now come respected authorities recommending large-scale efforts in tuberculosis prophylaxis using isoniazid.

In the April, 1960, issue of THE AMERICAN REVIEW OF RESPIRATORY DISEASES,[2] Dr. Harold L. Israel proposed isoniazid prophylaxis for ex-patients who have not had antituberculosis therapy and for an "additional 1,200,000 persons (who) had tuberculous lesions of doubtful clinical significance." In the same issue of THE REVIEW,[3] Dr. Walsh McDermott states that, "Dr. Israel . . . presents substantially the same conclusions as the (Arden House) conference." Dr. McDermott thus implies that the Arden House report recommends prophylaxis as described by Dr. Israel. This is not the case, however. In the Conference report under the heading, "Continue Isoniazid Studies," a very cautious wait and see attitude was expressed. This was quoted in part, above.

There are several studies which should influence our attitude toward drug therapy of ex-patients. It has long been known that relapses and reactivations in tuberculosis occur most frequently in the *early* years after therapy. This was especially notable before specific antimicrobials were available.

In the studies of Alling, Bosworth, and Lincoln,[4-6] relapse rates in the pre-chemotherapy era 1938–1948, were as follows in terms of years following diagnosis:

Per Cent of Relapses Occurring Annually

Years of Observation:	2	3	4	5	6	7	8	9	10
Patients Treated									
392 minimal cases	3.8	2.9	2.5	1.4	0.6	0	0	0	0
139 moderately advanced cases	6.5	6.9	2.1	2.8	3.1	3.8	0	?	?
61 far advanced cases	8.2	10.4	2.4	2.6	3.1	3.3	?	?	?

DeFriez and associates[7] found that in 377 patients, 95 initial relapses occurred during twenty years of observation (excluding 48 patients who died in the hospital from progressive disease). Of this number, 66 (69.5 per cent) relapsed in the first five years, 18 more (18.9 per cent) in the next five years, and only 11 (11.6 per cent) in the next ten years.

In two further studies, Mitchell[8, 9] found, in a large group of moderately and far advanced patients studied for twenty years, that 86 per cent of relapses had occurred by the end of five years. In a group of patients with minimal tuberculosis, 90 per cent of relapses had occurred within five years of beginning of treatment.

[1] Arden House Conference on Tuberculosis, Bull Nat Tuberc Ass, 1960, *46*, 22.

[2] ISRAEL, H. L.: Amer Rev Resp Dis, 1960, *80*, 581.

[3] MCDERMOTT, W.: Amer Rev Resp Dis, 1960, *80*, 579.

[4] LINCOLN, N. S., *et al.*: Amer Rev Tuberc, 1954, *70*, 15.

[5] ALLING, D. W., *et al.*: Amer Rev Tuberc, 1954, *70*, 995.

[6] ALLING, D. W., *et al.*: Amer Rev Tuberc, 1955, *71*, 519.

[7] DEFRIEZ, A. I., *et al.*: New Eng J Med, 1954, *250*, 39.

[8] MITCHELL, R. S.: Amer Rev Tuberc, 1953, *67*, 401.

[9] MITCHELL, R. S.: Amer Rev Tuberc, 1955, *72*, 487.

Finally, Mitchell[10] studied 115 patients who were reported as having primary relapse of tuberculosis *after* five years of arrest. Only 31 of the 115 had a history and roentgenograms consistent with stable disease during the five-year "arrest" period. It was the author's opinion that most of this group had smouldering disease or persistent cavity in areas of residual disease (before the days of planigraphic roentgenographic studies). In other patients, there was evidence that the "relapse" was caused by exogenous reinfection.

As virtually all inpatients for the past nine to ten years have received chemotherapy, the ex-patients who had no antituberculous drugs are almost all in a group that has already experienced most of its relapses. To enter into a broad program of prophylaxis as suggested by Israel and others would not only be productive of very small returns, but *would seriously detract from other more important aspects of the eradication effort* as proposed at Arden House.

Limited prophylaxis is already being carried out, and informed physicians are now prepared to use isoniazid alone or in combination for: (*a*) recent (within one year) tuberculin converters of any age, (*b*) infants under two or three years who are tuberculin reactors, and (*c*) individuals with newly discovered, small, roentgenographic lesions of uncertain activity when the skin test is positive and hospitalization is considered to be unnecessary.

Beyond this point, wishful thinking takes over. There is no scientific basis, or even a logical hypothesis, for the use of isoniazid prophylaxis for the huge group of tuberculin reactors in our population or the very large group of ex-patients treated before 1947–1948 who never received antituberculous drug therapy, or even those treated before 1952 who did not receive isoniazid as part of therapy.

No doubt there are some patients from the pre-drug era who have large pulmonary residues of previously active tuberculosis, and some who have roentgenographic or clinical evidence of unstable or smouldering disease, and still others whose life circumstances suggest the possibility of relapse. These should be considered individually for prophylactic chemotherapy, with a justifiable hope that relapse could be prevented.

In health jurisdictions where other recommendations of the Arden House Conference have been essentially fulfilled (and these are few), it would be worth while to promote pilot studies in broad-scale isoniazid prophylaxis. These will determine whether or not the incidence of new cases can be reduced by this method. Also the feasibility of giving daily medicine for a year or more to large groups of "well" persons will be tested. U. S. Public Health Service studies now in progress may report important values in isoniazid prophylaxis in selected groups. Only as such information becomes available, can we begin to assign relative values to the different facets of the prophylaxis problem.

In conclusion, it is well known that serious gaps exist in tuberculosis control programs in most parts of the country. It will take a maximal effort to repair these gaps. Until they are repaired, it would seem unwise to dissipate available energy by embarking on an unproved program of general isoniazid prophylaxis for tuberculin reactors and for expatients from the era before chemotherapy.

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April 28, 1960

[10] MITCHELL, R. S.: New Engl J Med, 1955, *253*, 640.

PHYSICAL THERAPY IN CHEST DISEASES

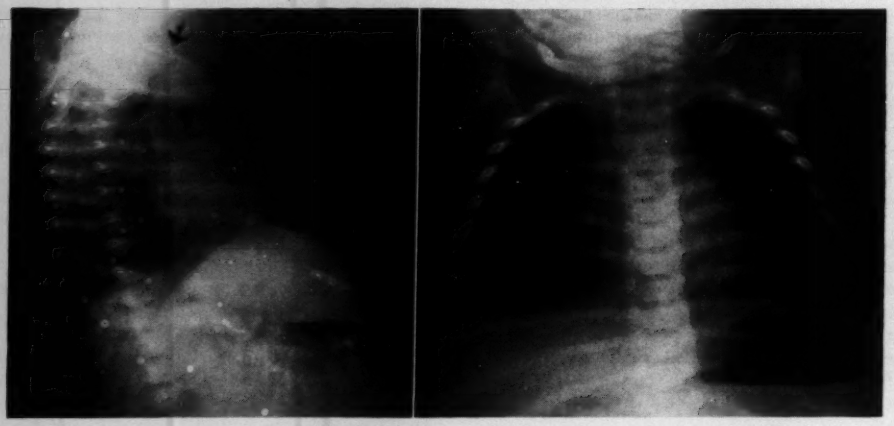
To the Editor of the American Review of Respiratory Diseases:

I was much interested to note in your May issue the comments of Drs. Harold R. Willard and Leonard Couture in their review of *Physical Therapy in Chest Diseases*, a book by Torstand Bruce, Carolyn Reutersward, and Brigit Westin.

The reviewers summarized that "the book makes a strong case for the use of physiotherapy in chest diseases. . . . However, in this country these valuable techniques are often overlooked."

I wish to note that there are in the United States today a number of fully planned rehabilitation programs for patients suffering from chest diseases and that these programs are well established and have real professional standards. The first such program, begun in 1949, was a cooperative effort made by Dr. Howard A. Rusk and the Chest Service of Bellevue Hospital. The experience of this Chest Rehabilitation Service has been described in numerous publications and lectures. The application of rehabilitative measures in chest diseases is also elaborated in an extensive chapter of Dr. Rusk's book *Rehabilitation Medicine* (published by C. V. Mosby). Further, the writer of this letter took part in initial plans for a chest rehabilitation program at the National Jewish Hospital, Denver, Colorado, that is now in full operation.

In my opinion, "these valuable techniques" have been less overlooked in the United States than the reviewers apparently believe. It is true that some of the underlying concepts have met with considerable skepticism. No efforts had been made until recently to test scientifically the value of such measures as diaphragmatic breathing exercises and postural drainage. Although such modalities were utilized, results were judged simply by the patients' subjective reaction or the bias of the treating physician. Skepticism was strengthened because many patients hospitalized for emphysema were in such a deteriorated condition that the only possible treatment was symptomatic.



Case No. 9778-60

FIG. 1 (Left). Patient before postural drainage.
FIG. 2 (Right). Patient after having postural drainage.

More careful clinical observation indicated a very real value to these modalities. A laboratory study was set up to substantiate these clinical findings. This research project, now in its fourth year, is being sponsored by the Office of Vocational Rehabilitation and is being carried out by the Department of Physical Medicine and Rehabilitation, New York University-Bellevue Medical Center. Data obtained to date on about 100 subjects indicated that, if patients are properly handled, results can be impressive. Pilot studies showed that spirometric measurements were too gross to record improvement of these patients. The apparent answer is the use of metabolic measurements. The investigation is utilizing measures of oxygen consumption during different activities, as well as measurement of oxygen debt and recovery rates. These methods have been

effective in demonstrating measurable improvement for the great majority of study patients.

Postural drainage coupled with administration of bronchodilator and detergents is followed in the study by positioning of patients to permit maximal gravity drainage. Use of these techniques, not only for emphysema patients, but also for those with other respiratory diseases, has been extremely effective.

Interesting is the history of a patient (L. R. Case #9778-60) referred to the Chest Rehabilitation Service by the Bellevue Pediatric Department.

A male infant of six weeks was admitted to the hospital on April 14, 1960, because of difficulty in breathing. The patient had seemed entirely well until three days before admission when he developed a cough and running nose. One day prior to admission, these symptoms worsened and breathing became laborious. An admission roentgenogram showed collapse of apical segments of the right upper lobe (figure 1). Until April 20, the patient showed no improvement. A roentgenogram taken April 20 revealed numerous opacities; it was concluded that there had been a collapse of the right upper lobe. The chest consultant suggested bronchoscopy. On April 21, the Chest Rehabilitation Service was consulted; postural drainage was prescribed and carried out three times daily. Drainage was obtained of relatively large amounts of secretion. A roentgenogram taken on May 2 (figure 2) showed normal re-expansion of the upper lobe, and the patient was discharged.

The treatment of this case is not unusual. Our experience lends credence to the opinion that physical therapy in the management of chest cases (especially postoperatively) is very well established and currently practiced in this country. Although some skepticism still prevails in regard to the value of breathing exercises and postural drainage, I doubt if many physicians now overlook the merit of physical medicine and rehabilitation for chest patients.

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May 24, 1960

RELIABILITY OF THE NIACIN TEST

To the Editor of the American Review of Respiratory Diseases:

The niacin test[1, 2] has been used widely to differentiate human tubercle bacilli from other mycobacteria, including unclassified mycobacteria, since it is a simple chemical test and can be used in small laboratories. Many confirmations and modifications of the procedure have appeared in the literature.[3-11] Some [3, 4, 10, 11] were confirmation of the results obtained by the original direct method and others[5-9] were modifications of that method.

Most of the writers[3-10] confirmed the usefulness of the niacin test, but Dr. M. L. Koch[11] claimed that the niacin test was too inconsistent and was not a reliable method of differentiating human tubercle bacilli from unclassified mycobacteria (Battey strain).

Accordingly, I would like to clarify and reaffirm the reliability and the usefulness of the niacin test.

As shown in table 1, the niacin test was tried on 1,084 strains of human tubercle bacilli and gave positive results on 1,060 (98 per cent) of the strains. Niacin-negative strains represented only 24 strains among the 1,084 strains of human tubercle bacilli or less than 2 per cent. Dr. Marek[3] observed 15 strains of niacin-negative human tubercle bacilli in his 406 strains, and he mentioned that these 15 strains were tested at the beginning of his experiment and that the aniline reagent used was too old for the test; therefore, the negativity of these 15 strains was incorrect. If these 15 strains were excluded from 24 niacin-negative strains, there would be only 9 niacin-negative strains against the 1,060 niacin-positive strains, and the positivity of the niacin test on human tubercle bacilli would be more than 99 per cent. On the contrary, Dr. Koch's results on the positivity of the niacin test on human tubercle bacilli was 70 per cent for 20 strains.

Among these 20 strains, H37Rv and H37Ra were tested as controls, and Dr. Koch complained that H37Ra gave a negative niacin reaction. I received H37Ra from her laboratory and retested for niacin. As shown in table 2, her H37Ra gave a positive qualitative niacin reaction, and the quantitative test showed also high niacin content in the culture filtrate. The Battey strain P-7 gave a negative niacin reaction and showed a much lower niacin content than H37Ra. In this laboratory, Dr. Koch's H37Ra strain definitely produces much higher niacin compared with the Battey strain P-7.

On this occasion I wish to recall the results of the niacin test on the unknown 26 strains of the Sunmount package.[2] In 1956, I received 26 strains, the identification of which was unknown to me, from the Subcommittee on Atypical Acid-fast Bacilli of the American Trudeau Society through Dr. E. H. Runyon. The niacin test was positive on 3 strains and negative on the other 23 strains. The 3 strains that were niacin positive were diagnosed as human tubercle bacilli, which was reported to the Subcommittee. The Subcommittee supplied the strain identifications of the unknown package, and the 3 niacin-positive strains were H37Rv, R1Rv, and H37Ra. Among 23 other niacin-negative strains, 3 were bovine, avian, and nonpathogenic and 20 strains were unclassified mycobacteria, including 7 Battey strains. Many biologic and biochemical tests were tried to identify these unknown 26 strains all over the world but, as far as the identification of human tubercle bacilli was concerned, the niacin test was the only test which could identify those 3 strains of human tubercle bacilli.

From the above facts and the more than 98 per cent positivity on 1,084 strains of human tubercle bacilli, it seems that the reliability of the niacin test on human tubercle bacilli is apparent.

Next, I wish to mention the reliability of the niacin test on other mycobacteria. As shown in table 1, the niacin test on 149 strains of bovine tubercle bacilli gave 93 per cent negativity. The 9 niacin test-positive strains were almost all laboratory strains. But these niacin-positive strains have very low nicotinamidase activity[12,13] and can thus be differentiated from other mycobacteria. All of 31 strains of avian tubercle bacilli gave negative niacin reactions, and 105 strains of sapro-

[1] KONNO, K.: Science, 1956, *124*, 985.

[2] KONNO, K., *et al.*: Amer Rev Tuberc, 1958, 77, 669; 675; and 1958, *79*, 810.

[3] MAREK, A.: Beitr Klin Tuberk, 1957, *117*, 529.

[4] BOENICKE, R.: Jahresbericht Borstel, 1956/57, p. 43.

[5] JENSEN, K. A., AND KIAER, I.: Bull Un Int Tuberc., 1958, *28*, 156.

[6] GILANI, S., AND SELKON, J. B.: Tubercle, 1958, *39*, 396.

[7] GUTIERREZ-VAZQUEZ, J. M.: Rev Lat Amer Microbiol, 1958, *1*, 287.

[8] RUNYON, E. H., *et al.*: Amer Rev Tuberc, 1959, *79*, 663.

[9] BESTA, B.: Bull Un Int Tuberc, 1959, *29*, 308.

[10] TOMODA, T., *et al.*: Kokyuki Shinryo (in Japanese), 1960, *15*, 96.

[11] KOCH, M. L., AND KROLL, A. W.: Amer Rev Resp Dis, 1960, *81*, 108.

[12] KONNO, K., *et al.*: Nature, 1959, *184*, 1743.

[13] KONNO, K., *et al.*: Amer Rev Resp Dis, 1960, *81*, 550.

TABLE 1

RESULTS OF THE CONFIRMATION AND THE MODIFICATION STUDIES OF THE NIACIN TEST

Authors	Country	Human Tubercle Bacilli			Bovine Tubercle Bacilli			Avian Tubercle Bacilli			Unclassified Mycobacteria			Saprophytic Mycobacteria		
		Strains	Niacin + Strains	Per Cent	Strains	Niacin + Strains	Per Cent	Strains	Niacin − Strains	Per Cent	Strains	Niacin − Strains	Per Cent	Strains	Niacin − Strains	Per Cent
Marek, A.[3] (1957)	Austria	406	391	96	66	59	89	4	4	100	12	12	100	7	7	100
Boenicke, R.[4] (1957)	Germany	86	86	100	61	61	100	12	12	100	28	28	100	71	71	100
Jensen, K. A., and Kiaer, I.[5] (1958)	Denmark	242	238	98	10	10	100	1	1	100	17	17	100	14	14	100
Gilani, S., and Selkon, J. B.[6] (1958)	England	13	13	100	5	5	100	1	1	100	5	5	100	1	1	100
Gutierrez-Vazquez, J. M.[7] (1958)	Mexico	12	12	100	1	1	100	2	2	100	6	6	100	12*	10	83
Runyon, E.[8] (1959)	United States	166	162	98	3	1 (2±)	33	6	6	100	49	49	100			
Besta, B.[9] (1959)	Italy	20	20	100		67					14	14	100			
Tomoda, T. *et al.*[10] (1960)	Japan	139	138	99	3	3	100	5	5	100	1	1	100			
Total		1084	1060	98	149	140	94	31	31	100	132	132	100	105	103	98
Koch, M. L. *et al.*[11] (1960)	United States	20	14	70							115	99	86			

* Including 1 strain of *M. microti*, *M. ulcerans*, *M. balnei*, *M. marinum*

TABLE 2

RESULTS OF NIACIN TEST ON DR. KOCH'S NIACIN TEST-NEGATIVE H37Ra AND BATTEY STRAIN (P-7)

Strains	Qualitative Niacin Test: Egg Medium	Quantitative Niacin Test (Niacin γ/ml.), Sauton Liquid Medium
Dr. Koch's H37Ra	+	38.2
Battey strain P-7	−	2.4

phytic mycobacteria gave 98 per cent negative niacin reactions.

Of the unclassified mycobacteria, Dr. Koch reported that many of her Battey strains gave positive niacin reactions. She tested 115 strains, and 16 strains gave positive reactions. Eight investigators in Europe and America tested altogether 132 strains of unclassified mycobacteria and all were niacin negative, including Runyon's 15 Battey strains. This represents 100 per cent negativity on the niacin test. At the XVth International Tuberculosis Conference, held in Istanbul, Turkey, in 1959, the niacin test was recommended as one of the methods to differentiate human tubercle bacilli from unclassified mycobacteria.

TABLE 3

ISOLATION AND NIACIN TEST OF TYPICAL AND UNCLASSIFIED MYCOBACTERIA FROM TUBERCULOUS PATIENTS IN JAPAN

		per cent
Typical colonies, niacin-test positive	567	84.0
Typical colonies, not enough growth for niacin test	65	9.6
Unclassified mycobacteria		
Niacin-test negative		
Photochromogens	0	0
Scotochromogens	23	3.4
Nonphotochromogens	20	3.0
Total	675	100.0

In Japan, the Committee on the Mutation and the Classification of Mycobacteria has been studying the Japanese strains and the United States strains of unclassified mycobacteria. Among the Japanese unclassified strains, 2 strains[14, 15] (Battey type) gave positive niacin

[14] URABE, K., AND KAWAI, Y.: Medicine and Biology (in Japanese), 1957, *42*, 33.

[15] URABE, K., AND KAWAI, Y.: Medicine and Biology (in Japanese), 1958, *47*, 143.

reactions. These 2 strains were thought to be unclassified mycobacteria that were pathogenic for humans. These 2 strains were studied by the Committee on animal pathogenicity, mycobacterial phage typing, and the susceptibility to chemotherapeutic agents. All studies revealed that these strains were human tubercle bacilli and not unclassified mycobacteria and, therefore, these 2 strains were identified as human tubercle bacilli.[16]

I wish to point out that there were human tubercle bacilli among "so-called atypical mycobacteria" which could be distinguished by the niacin test.

In Japan, the unclassified mycobacterial infection is rather rare compared with that in the United States; photochromogenic mycobacterial infection is extremely rare. I could not find any photochromogens in our Institute among 675 positive cultures obtained from the sputum or gastric lavages of tuberculous patients (table 3). Among 675 strains, 43 strains (6.4 per cent) of unclassified mycobacteria were found, and all of the strains were niacin negative. Five hundred and sixty-six strains (84 per cent) of tubercle bacilli were niacin positive. Sixty-five strains (9.6 per cent) did not grow enough on subculture for a niacin test. Konno and associates[2] stated that more than 10 mg. of bacilli were necessary to perform the test on solid medium, and Marek[3] raised this figure to 20 mg.

On performing the niacin test on the solid medium, the most important thing is to secure enough bacilli, which should be more than 10 mg. wet weight. The niacin test can be carried out as follows: One milliliter of distilled water is added to the culture tube. The water in the tube is heated by gas burner, facing the culture surface down, and the niacin of the bacteria is extracted. Then, the culture is placed horizontally for a few minutes; the water is divided into two parts.[17] Each 0.1 ml. of 4 per cent aniline ethanol solution and 10 per cent of aqueous cyanogen bromide are added to one part; only aniline solution is added to the control. Only human tubercle bacilli will give a yellow color.

The investigations on the reliability of the niacin test in the various parts of the world, except those of Dr. M. L. Koch, revealed 98 per cent positivity on 1,084 strains of human tubercle bacilli, 94 to 100 per cent negativity on other mycobacteria. The niacin test seems to be reliable as a simple chemical test to differentiate human tubercle bacilli from other mycobacteria.

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June 8, 1960

[16] TAKEYA, K.: Annual Meeting of the Japanese Tuberculosis Association, 1960.

[17] Recent modification of the niacin test (E. Medveczky, Amer Rev Resp Dis, 1960, *81*, 757) using 3 per cent benzidine ethanol solution and 10 per cent aqueous cyanogen bromide, can be carried out on the water used to wash the bacteria. The water is divided into four parts: two parts are used for the aniline–cyanogen bromide method described above; the other two parts are used for the benzidine-cyanogen bromide method. Each 0.1 ml. of the benzidine solution and the cyanogen–bromide solution is added to one part; benzidine solution is added to the control. Only human tubercle bacilli will give a pink color precipitation, while other mycobacteria and the controls remain a white precipitation. Concomitant use of the benzidine–cyanogen bromide method with the aniline–cyanogen brōmide method will give more satisfactory results.

Books

RECENT STUDIES IN EPIDEMIOLOGY. Edited by J. Pemberton and H. Willard. Pp. 203, Charles C Thomas Company, Springfield, Illinois, 1958. Price, $5.75.

This series of eighteen papers, presented at the 1957 meeting of the Study Group on Current Epidemiological Research, comprises a fascinating record of the diverse areas in which the epidemiologic approach can be used with profit. Reported by eminent epidemiologists from eight countries of Europe and North America, these studies demonstrate the diversity of areas in which the epidemiologic approach may be used as well as the suitability of this method to clarify problems needing further study. Most of the studies discussed are concerned with noncommunicable diseases, but consideration is also given to the somewhat different approaches needed for communicable disease problems, particularly in underdeveloped areas of the world.

The most valuable contribution of this volume is the emphasis on method, which is a prominent feature of every paper included. In general, the studies presented fall into one of three classifications. First are those studies based on the use of existing records of various types. The difficulties inherent in such studies are too well known to need further comment, but the interesting conclusion is drawn that increasing use of existing records for research purposes should lead to an improvement in the quality of medical records. If this should be the case, all who work with public health records will be grateful to those prompting such changes. Second are those studies based on ad hoc field surveys, in which the data were collected by the survey team. A variety of different approaches is used by the various investigators in such diverse areas as coronary artery disease, motor vehicle accidents, infectious hepatitis, and mental illness.

Third is an area too much neglected by epidemiologists—the use of this discipline for evaluation of public health needs and services as well as for judging the effect of public health measures in solution of disease problems. Three papers illustrating this use of epidemiologic methods should be of particular value to the public health worker in the field, as well as to the researcher.

Another problem of increasing public health importance, which is well covered in this useful volume, is that of a disease population shading imperceptibly into an unaffected group without sharp distinctions between them. Papers on studies in such fields as bronchitis and coronary artery disease illustrate the problems of research workers in this area and suggest some ingenious solutions.

Although the principal emphasis of the book is on the uses of epidemiology in research, an interesting appendix includes the opinions of the members of the study group on the teaching of preventive and social medicine. Teaching objectives in this field appear to vary considerably between and within countries, depending primarily on local needs and facilities. Teaching methods vary even more widely, and here the principal problem appears to be how to integrate preventive and social medicine with patient care. There are marked differences between those who believe this implies a separate discipline and those who believe no attempt should be made to cover these areas by themselves but, rather, that they should be absorbed into the general discipline of clinical medicine.

In summary, this little volume provides a remarkably comprehensive survey of the ways in which epidemiologic methods are being used in different parts of the world under a variety of local conditions. It will provide both pleasure and profit to all involved in community research projects as well as to those interested in the preventive and social aspects of medical care.

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THE YEAR BOOK OF MEDICINE—1959–1960. Edited by Paul B. Beeson, Carl Muschenheim, William B. Castle, Tinsley R. Harrison, Franz J. Ingelfinger, and Phillip K. Bondy. Pp. 733, The Year Book Publishers, Inc., Chicago, 1959. Price, $8.00.

This volume contains summaries of 539 articles covering recent advances in internal medicine. The articles abstracted appeared in clinical journals during the past year and were selected by the board of six distinguished editors. The book is divided into six sections: Infections (Beeson), The Chest (Muschenheim), The Blood and Blood Forming Organs (Castle), The Heart and Blood Vessels and The Kidney (Harrison), The Digestive System (Ingelfinger), and Metabolism (Bondy). One of the most valuable features of *The Year Book*

of Medicine is the editors' comments following many of the articles. *The Year Book* is recommended as a worth while review of recent literature and current thought in the field of internal medicine.

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THE YEAR BOOK OF GENERAL SURGERY, 1959–1960. Edited by Michael E. DeBakey. Pp. 639, The Year Book Publishers, Inc., Chicago, 1959. Price, $8.00.

The Year Book of Surgery has become a valuable reference source in both the teaching and practice of surgery. The current volume is particularly outstanding and qualifies as the finest yet published in the series. A total of 448 papers have been selected for review and represent an excellent coverage of a variety of subjects. A number of journals have been used as sources of the material, and pertinent papers from nonsurgical publications have been judiciously chosen to broaden the value of the collection. Perhaps the most significant contribution is to be found in the concise and authoritative notes by the Editor which follow many of the abstracts. These comments are quite helpful in offering the reader a reliable source of endorsement or an alternative point of view. This aspect of the *Year Book* emphasizes the fact that the editorship of a work of this scope requires a broadly based background in the teaching and training of surgical principles and practice. It could not have been more admirably performed than is done in the present volume.

In conclusion, it may be said with confidence that the current volume is an excellent source of the recent contributions to surgery. It is highly recommended as a valuable companion for the surgical practitioner, teacher, and investigator.

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RESPIRATORY PHYSIOLOGY AND ITS CLINICAL APPLICATION. By John H. Knowles. Pp. 256, Harvard University Press, Cambridge, Massachusetts, 1959. Price, $5.25.

This small book, intended for the medical student and practicing physician, presents physiologic concepts in a clear and concise manner. Dr. Knowles has succeeded in removing much of the mystery of respiratory physiology by avoiding many of the technical terms and formulas of the specialist without compromising clarity. The book is divided into two parts: in the first, he discusses lung volumes, ventilation, distribution of gases, diffusion, the work of breathing, and blood gases. The second part is concerned with a few conditions which have been elucidated by studies of function, such as emphysema, poliomyelitis, and air cysts. In each instance there is a brief description of the clinical features, a section on the pathologic physiology, and a discussion of the results of studies of function.

The book focuses on respiratory physiology; the clinical applications are highly selected to illustrate aspects of disease capable of clarification by study of function. This approach is useful in indicating types of derangement which may occur in a host of diseases. It will prove disappointing to anyone expecting to find a compendium of respiratory diseases and a guide to suitable tests. For example, there is only an occasional reference to bronchial tumor, and this is not in the index. On the other hand, one chapter is devoted to polycythemia vera, one to idiopathic alveolar hypoventilation, and another to pneumonectomy. The author has clearly made no attempt to discuss all disease states in which pulmonary function studies have been done. One wonders what prompted him to select for emphasis the conditions he chose unless, perhaps, it is the amount of literature devoted to each condition.

In simplifying many complicated aspects of respiratory physiology, Dr. Knowles has tended to make short, positive statements, many of which need qualification. One could take exception to: "functional effects of pregnancy, ascites, and pneumoperitoneum are similar and result from a common process, namely abdominal distention." In the next paragraph he says that the dyspnea of pregnancy cannot be explained on the basis of reduced ventilatory ability. Instead of discussing the interesting problem of hyperventilation in pregnancy, he elaborates the extent of changes in lung volumes and compares it with ascites and pneumoperitoneum, in which the dyspnea may be accounted for on lung volume changes. Fortunately, the references are ample and well chosen, so that the interested reader may quickly find the qualifications he wants.

It is unfortunate that there are only eight illustrations. The ones included are well chosen, such as the oxygen dissociation curve and Fenn's pressure-volume curves of the human chest. There are fourteen tables which add greatly to the usefulness of the book.

This book is recommended to the clinician as an introduction to aspects of pulmonary physiology which have pertinence in the diagnosis and management of chest disease.

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BOOKS RECEIVED

FROM FISH TO PHILOSOPHER. The Story of Our Internal Environment. By Homer W. Smith. Pp. 304, issued by Ciba Pharmaceutical Products, Inc., Summit, New Jersey, 1959.

SUVREMENE TEME IZ PNEUMOFTIZEOLOGIJE. Edited by Mile Budak. Pp. 248, Zastita Zdravlja, Ustanova za Zdravstvenu Propapandu, Zagreb, Yugoslavia, 1960.

STUDIES ON LUNG MECHANICS. By P. G. Donleben. Pp. 164, Drukkerij Romijn, Apeldoorn, The Netherlands, 1959.

CAUSES OF COUGHING IN A GRONINGEN GENERAL PRACTICE. By Alfred Löwenberg. Pp. 291, Drukkerij Van Denderen, Groningen, The Netherlands, 1959.

THE HUMAN LUNG. By Heinrich von Hayek. (Translated by Vernon E. Krahl) Pp. 372, Hafner Publishing Company, Inc., New York, 1960. Price, $13.50.

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THE THYROID-VITAMIN APPROACH TO CHOLESTEROL ATHEROMATOSIS AND CHRONIC DISEASE. A Ten-Year Study. By Murray Israel. Pp. 132, The George Press, Inc., New York, New York, 1960.

DIE SUBMIKROSKOPISCHE ANATOMIE UND PATHOLOGIE DER LUNGE. By Heribert Schulz. Pp. 199, Springer-Verlag, Berlin, Göttingen, Heidelberg, 1959. Price, DM 178.

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DIE ASBESTOSE DER LUNGEN. Genese—Klinik—Rontgenologie. By H. Bohlig, G. Jacob, and H. Müller. Pp. 166, Georg Thieme Verlag, Stuttgart, 1960. Price, $15.70.

American Thoracic Society

Medical Section of the National Tuberculosis Association

Acute Respiratory Diseases

Report to the Council of the American Thoracic (Trudeau) Society of the *Ad Hoc* Technical Committee on Acute Respiratory Diseases (May 15, 1960)

The committee believes that, in the field of acute respiratory diseases, the American Thoracic (Trudeau) Society and its parent organization, the National Tuberculosis Association, can best serve the public *at this time* by fostering a program designed to educate the public and the medical profession about the nature of the viral infections of the respiratory tract, their prevention, and their treatment (special emphasis on mistreatment). The following outline of some opinions of this committee is submitted to the American Thoracic Society with a suggestion that it also be transmitted to the National Tuberculosis Association and the National Conference of Tuberculosis Workers.

The promotion of laboratory and epidemiologic research is regarded as a prime need, and it is believed that great advances should be anticipated during this decade. This report concerns practical and clinical aspects of some current problems, rather than research developments and needs.

Influenza

Influenza is a very specific group of closely related diseases and should be sharply distinguished from the "flu syndrome," a term often used carelessly by patients and physicians. True influenza can be diagnosed only by virologic laboratory procedures. They are not generally applicable to the needs of the individual patient, but are of great value for the identification of causes of epidemics. The clinical (symptomatic) identification of true influenza is fairly accurate during epidemics, but extremely inaccurate at other times since so many disorders may simulate influenza symptomatically.

Treatment of influenza is entirely protective and symptomatic, and the tendency to overtreat should be discouraged. Antimicobials are of no value and usually are not advisable even for protection against bacterial complications. Early isolation of an individual patient may limit family epidemics and avoid wider dissemination of the infection.

Vaccination against influenza offers excellent protection to the individual for a period of at least several months. The protection begins to be effective about two weeks after the initial injection. Immunity is rather sharply type-specific, and polyvalent vaccines are often recommended, but with recognition of the fact that future epidemics may be due to strains which differ from those causing preceding recent epidemics. Improvements in vaccination methods may result from research now in progress.

Mass vaccination of entire large populations against influenza is often not feasible because the epidemic is likely to spread more rapidly than type-specific vaccination can be accomplished, and pandemics are so infrequent that continuous universal protection is not a practical goal.

Routine vaccination, once each year, is recommended for certain special-risk groups, including patients with certain chronic respiratory diseases such as chronic generalized obstructive pulmonary emphysema, bronchial asthma, unstable pulmonary tuberculosis, severe bronchiectasis, and severe chronic bronchitis. The need for vaccination is increased when ventilatory insufficiency is considerable, when the person is in an advanced age group, during pregnancy, and when previous experience has demonstrated a poor tolerance of the individual for acute respiratory infections. Persons with severe cardiac insufficiency, especially those who have been decompensated and those who have mitral stenosis, should be vaccinated.

Para-Influenza Viral Infections

These infections are especially prevalent among small children and are reported to cause up to 70 per cent of the cases of the severe "croup" syndrome in infants, although other viral infections can produce similar acute obstructive laryngeal tracheobronchitis. Clinical manifestations of the para-influenzal viral infections vary widely. There is no vaccine available at this time and it is not known if conditions will justify production and widespread use of a vaccine, although it is believed that artificial immunization can be achieved.

Adenoviruses

This group of viral agents is a very large one (at least 27 types are known) with varying pathogenicity and clinical manifestations. They are often encountered in children and in military personnel, but probably cause only a small proportion of the cases of clinical respiratory tract infections among adults. It is the opinion of this committee that any vaccine now available for use against the respiratory adenoviruses cannot be recommended for general use to prevent acute respiratory infections.

Measles

Measles is truly a respiratory disease and of great medical significance. Very possibly the American Thoracic Society—National Tuberculosis Association should be concerned with this infection. The committee suggests that the matter be considered thoroughly.

Anti-measles vaccines now under study show promise, and it is probable that artificial immunizaion may become practicable. First, the vaccine must be perfected (stability, potency, standardization, et cetera). Next, a well-organized study of a closed group of susceptible persons must be completed. Finally, mass testing will be required. It is possible that the tuberculosis associations might serve well in promoting this latter project.

The Common Cold

Health education efforts are badly needed to dispel common misconceptions about this group of diseases (infection versus exposure, antimicrobials not indicated, avoidance of proprietary remedies, confusion with more serious diseases, et cetera).

It is believed that numerous viral agents—perhaps 50–70, even more—are capable of causing symptoms resembling those of the common cold. Some of the most prevalent viral agents have not been identified and cultured (recent reports from England indicate a possible "break-through" on this subject).

There is no available vaccine which can be recommended by this committee for prevention of the common cold. Vaccines now available are directed against adenoviral infections found to be prevalent in military personnel but believed by the committee to be rare in the general adult population. It is believed that future developments will make available vaccines effective against multiple viruses, including some related to "common colds."

Treatments designed for relief of common cold symptoms rarely provide benefits, and some are capable of harm when used injudiciously. Physicians should resist requests by patients for antimicrobial prescriptions and use this opportunity to inform their patients about some facts concerning these diseases.

Transmission of Viral Infections

There is much uncertainty about the relative importance of: (*a*) inhaled moist secretion droplets, (*b*) inhaled air-borne dry residuals of dessicated droplets, (*c*) hand-to-mouth transmission, and (*d*) unusual routes such as tableware, swimming pools, et cetera, as routes of transmission for respiratory viruses.

The epidemiology of these diseases is a complicated and incompletely understood subject. Many family epidemics of the common cold probably originate from school children and close association between ill and well children in the schools which may result in pooling of infections and subsequent dissemination. It is not known if this could be prevented by keeping children home when they have symptoms and if less stress were placed upon attendance records in elementary and secondary schools.

If temperature and other weather conditions are related to the common cold, it is a remote relation and one greatly exaggerated among the general population.

Many attempts have been made to purify air by radiation, filtration, or chemical sterilization. Practical and widespread application of these methods to prevent transmission of viral infections has not been accomplished, but there is reason to hope that controlled air hygiene may eventually provide some protection under special circumstances. Effects of atmospheric pollution on respiratory infections are not known.

John Dingle
Harry F. Dowling
Robert H. Ebert
Thomas Francis, Jr.
Robert J. Huebner
H. Corwin Hinshaw, *Chairman*

NOTICES

The following dates and locations of the next four annual meetings of the National Tuberculosis Association and the American Thoracic Society have been selected and approved:

1961—Cincinnati, Ohio—May 21–26
1962—Miami Beach, Florida—May 20–25
1963—Denver, Colorado—May 12–17
1964—New York, New York—May 24–29

1961 ANNUAL MEETING OF THE AMERICAN THORACIC SOCIETY

INVITATION

The Medical Sessions Committee *INVITES SUBMISSION OF PAPERS* on all scientific aspects of tuberculosis and nontuberculous respiratory and cardiopulmonary diseases for presentation at the *1961 ANNUAL MEETING IN CINCINNATI, OHIO, MAY 22–24.*

ABSTRACTS

(*1*) Abstracts of papers to be offered for presentation should be submitted before January 7, 1961 to the Chairman of the Medical Sessions Committee. *EIGHT COPIES* are required for distribution to committee members.

(*2*) Abstracts should be limited to a 300-word summary including *IMPORTANT DATA AND CONCLUSIONS.* Charts and tables should be attached to aid in the selection of papers. Abstracts selected for presentation at the Annual Meeting will be published in THE AMERICAN REVIEW OF RESPIRATORY DISEASES.

(*3*) Reports of interesting or unusual cases will be considered for inclusion in case conferences.

(*4*) The names, academic degrees, titles, and complete addresses of the senior author and co-authors should be included in each abstract.

(*5*) All abstracts will be reviewed by the Medical Sessions Committee. Selection will be largely based on the areas of interest suggested by you and others receiving this invitation.

(*6*) Authors will be notified by February 20, 1961, if their papers have been accepted for presentation.

(7) Papers presented at the Annual Meeting must be *ORIGINAL* contributions *NOT PREVIOUSLY PRESENTED OR PUBLISHED.* Medical papers presented at the meeting may be submitted to THE AMERICAN REVIEW OF RESPIRATORY DISEASES by the author if he so desires.

SUGGESTIONS

The Medical Sessions Committee is particularly anxious to build the 1961 program around your special interests. A memorandum suggesting subjects and speakers would be welcome and should be sent to the Chairman by September 20, 1960.

L. H. Schmidt, Ph.D., *Chairman*
Medical Sessions Committee
American Thoracic Society
1790 Broadway, New York 19, N. Y.

NATIONAL TUBERCULOSIS ASSOCIATION FELLOWSHIPS

As the Medical Section of the National Tuberculosis Association, the American Thoracic Society (formerly the American Trudeau Society) provides fellowships and traineeships for the graduate education of investigators and teachers in the field of respiratory diseases and tuberculosis. The following types are offered:

Edward Livingston Trudeau Fellowships: A few fellowships at a higher level of training and award are offered to specially qualified candidates with an M.D. degree who have been assured of a continued teaching or research appointment upon completion of training. Trudeau Fellowships are awarded for one year, but may be renewed up to a total period of four years.

Research Fellowships: Postdoctoral fellowships are offered to candidates holding the degree of M.D., Ph.D., or Sc.D. for further training as scientific investigators.

Predoctoral fellowships are offered to graduate students with a bachelor's degree to work on a research project for an advanced degree other than M.D.

Teaching Traineeships: Physicians entering their second or third year of residency in Internal Medicine or Thoracic Surgery at a medical center who may be interested in a career of medical teaching are offered traineeships toward that end. Supplementation of these awards by the institution involved is not necessary.

Each applicant for an NTA fellowship or traineeship must have been accepted by the head of the department under whom he expects to work for the next academic year in this country. Awards are determined by individual circumstances and are paid directly to the Fellow on a quarterly basis. They are granted for one year, beginning on any date after April first, and are renewable for a total of three years.

All applications must be submitted by November 1st. Further information about Fellowships may be obtained upon request from:

Director of Medical Education
American Thoracic Society
1790 Broadway
New York 19, New York

TERATOLOGY SOCIETY

For several years, scientists interested in basic problems of congenital malformations have held informal conferences in which questions of common interest were discussed. Anatomists, biochemists, embryologists, geneticists, obstetricians, pathologists, pediatricians, plastic surgeons, and others attended these conferences, which were in part supported by the Association for the Aid of Crippled Children, New York, New York, and the Human Embryology and Development Study Section of the National Institutes of Health. With the increased interest in this area in recent years, it was believed that there was a need for a Society to hold regular meetings in which investigations concerned with the etiology and morphogenesis of congenital malformations could be presented and discussed. Following the fourth teratology conference, which was held at the Memorial Sloan-Kettering Cancer Center in New York City, and attended by seventy-six scientists from Canada, England, France, Germany, and the U.S.A., "The Teratology Society" was formed for the purposes outlined above. The following officers were elected: President: Josef Warkany, M.D., Cincinnati, O.; President-Elect: James G. Wilson, Ph.D., Gainesville, Fla.; Secretary-Treasurer: Marjorie M. Nelson, Ph.D., San Francisco, Calif.; Recorder: Sidney Q. Cohlan, M.D., New York, N. Y.; Council: F. Clarke Fraser, Ph.D., M.D., Montreal, Canada; David L. Gunberg, Ph.D., Portland, Ore.; and M. Lois Murphy, M.D., New York, N. Y. The National Foundation assisted in the formation of the Society with advice and financial aid. Inquiries about the Teratology Society should be directed to Dr. Marjorie M. Nelson, Department of Anatomy, School of Medicine, University of California, San Francisco 22, California.

COURSE IN LABORATORY METHODS IN THE DIAGNOSIS OF TUBERCULOSIS

In cooperation with the Division of Special Health Services, Public Health Service, the Microbiology Laboratories of the Communicable Disease Center, Atlanta, Georgia, will offer a course in Laboratory Methods in the Diagnosis of Tuberculosis on the following dates:

October 31–November 11, 1960
January 30–February 10, 1961

This course is open to all grades of employed laboratory personnel who are approved by their State Health Officers. Practical laboratory training in all phases of tuberculosis bacteriology, including preparation of culture media, microscopy, cultural procedures, diagnostic use of animals, cytochemical techniques, and testing of drug susceptibility will be included. Demonstration and training in techniques for the isolation and identification of unclassified acid-fast bacilli will also be presented.

Laboratory directors and senior laboratory staff members may also make application for this course. No tuition or laboratory fees are charged. Reservations for this course should be made well in advance. Application forms may be obtained from the Laboratory Branch, Communicable Disease Center, Public Health Service, Atlanta, Georgia.

THE AMERICAN REVIEW
OF RESPIRATORY DISEASES

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

ABSTRACTS

Edited by EDWIN D. KILBOURNE
Editorial Assistant: TERRI MANN

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The Editor recognizes the inevitable incompleteness and arbitrary selectivity of any abstracting service, including this one. It is his belief that the usefulness of the present Abstracts section will be enhanced if the reader is informed of the general purpose of the abstracts and the criteria employed in their selection.

It is the purpose of the Abstracts section to acquaint the diversified and international readership of the REVIEW with the gist of original and scientific articles which are related to the general subjects of tuberculosis or respiratory diseases implicit in the title of the journal. In this connection, 42 abstracters systematically review 239 journals which represent 36 countries and which are published in 22 languages. In most instances, abstracters cover journals in fields of their special competence. It is evident that the diverse interests of the readers of the REVIEW dictate a balanced selection of clinical, laboratory, and epidemiologic studies. Such selection will often preclude the publication of articles equal in scientific merit to many presented here.

In the publication of abstracts, cognizance is taken of the extent to which the journals yielding them are circulated. Articles from journals of wide circulation may be mentioned by title only. This space-saving device is also employed for articles which defy condensation without the omission of essential data or discussion. The attention of readers is thus directed to the existence of papers which are no less important because they are not summarized.

(Requests for further information on abstracted articles should be directed to the author of the article at the address indicated by an asterisk (*) in each abstract title.)

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NONTUBERCULOUS THORACO-PULMONARY DISEASE

Acute Bronchial Asthma. I. Antecedents. P. KNAPP* and J. NEMETZ. *Psychosom Med*, January–February, 1960, 22: 42–55. (*Boston U. School of Med., Mass.)

Based on 406 asthmatic attacks in 9 patients with severe chronic bronchial asthma who were investigated by psychiatric or (in 2 patients) psychoanalytic techniques, some of the emotional concomitants and underlying factors in asthma are presented. Feelings and fantasies concomitant with the attack were dominated by absorption in the physical illness. In more than half of the attacks, there was also a strong element of depression. Occasionally there were fantasies of a dangerous or poisonous inner substance. In somewhat less than half of the attacks there were prodromal emotional manifestations, usually angry or anxious, but occasionally elated or erotic. Antecedent changes in the emotional environment were widely varied, but most commonly were related to loss of, or closeness to, a person. It was postulated that either loss of or closeness to a person might activate a respiratory apparatus, having both intake and expulsion functions, which has become vulnerable to psychologic as well as physiologic stress.—E. J. CASSELL

The Pathology of Asthma, with Special Reference to Changes in the Bronchial Mucosa. M. S. DUNNILL*. *J Clin Path*, January, 1960, 13: 27–36. (*Radcliffe Infirmary, Oxford, England)

The pathologic features of 20 patients who died in status asthmaticus have been studied. In gross sections the lungs showed no emphysema, but mucous plugs in the air passages and focal areas of collapse were outstanding features. Five cases showed cystic bronchiectasis which was of a similar distribution to the focal areas of collapse, occurring in the upper lobes as commonly as in the lower lobes. Histologically, shedding of the ciliated bronchial mucosal cells was prominent; this is attributed to a transudation of edema fluid from the submucosa. Areas of regeneration of the mucosa, with the presence of simple stratified epithelium, were seen frequently. The loss of the ciliated respiratory epithelium and the transudation of edema fluid into the bronchial lumen, with interference with the action of the remaining ciliated cells, are considered to be the essential factors in the failure of clearance of the bronchial secretions in asthma. It is postulated that bronchospasm plays little or no part in the shedding of the bronchial mucosa or in the pathogenesis of the asthmatic attack.—E. DUNNER

Diagnosis and Treatment of Bronchial Asthma in Childhood (in German). F. WORTMANN*. *Schweiz Med Wschr*, September 26, 1959, 89: 1014–1017. (*Universitätsklinik, Basel, Switzerland)

The first symptoms of bronchial asthma in 517 children appeared mainly before the age of 3 years. The beginning was often characterized by repeated attacks of common colds or of so-called asthmatic bronchitis. The scratch test with different commercially available house-dust extracts was negative in 25 per cent of the patients, although they had a strong positive reaction to the house dust from their own surroundings. Therefore, tests with dust samples of the patients' own homes should always be included in the routine testing of asthmatics. Examination of the nasal smear for eosinophils offered an easy and dependable diagnostic aid in the differentiation between allergic and nonallergic respiratory affections. The specific desensitization should be made with extracts from the patient's own house dust. Oral desensitization with house-dust extracts gave good results in children up to the age of 12–14 years.—J. HAAPANEN

Eosinophilia in Children with Asthma and Bronchiectasis. L. B. STRANG*. *Brit Med J*, January 16, 1960, No. 5167: 167–169. (*U. of Durham, England)

Eosinophil counts were performed on 40 children with bronchiectasis and 43 with asthma. Eosinophilia of more than 600 per cu. mm. was present in 37 of 43 patients with asthma, and in none of 40 with bronchiectasis. The degree of eosinophilia was generally unrelated to the severity of the asthma, and was frequently present between attacks. The presence or absence of eosinophilia may serve as a means of distinguishing these conditions in borderline cases.—E. A. RILEY

Lung Cancer in Coal Miners. R. A. SMITH*. *Brit J Industr Med*, September, 1959, 16: 318–323. (* King Edward VII Memorial Chest Hosp., Warwick, England)

In a study of coal miners suffering from lung cancer, two features of special interest are recorded. The difficulties in diagnosis are illustrated by case reports. The 2-year survival rate after surgical removal is significantly better in miners than in non-miners. After operation, 87 per cent of coal miners were alive 2 years later, compared to only 36 per cent of other patients operated upon. It is suggested that the rate of spread of the tumor to the mediastinal structures, lymph nodes, and blood vessels is retarded by the effects of pre-existing dust disease in the lungs. This factor explains the good results in the patients selected for surgery (after author's summary).—H. SIMON

Chronic Cor Pulmonale Due to Multiple Pulmonary Emboli and Accompanied by Diffuse Interstitial Fibrosis. S. LANGFIELD*, T. HOPKINS, and E. THEURKAUF, JR. *Amer J Med*, September, 1959, 27: 494–500. (* U. of Pennsylvania School of Med., Philadelphia)

There are frequent reports in the literature of patients with multiple pulmonary emboli ultimately dying from right ventricular failure. The case reported is of interest because of clinical features which required extensive diagnostic studies, and the infrequently reported finding of diffuse interstitial fibrosis of the lungs accompanying multiple pulmonary emboli.—T. H. NOEHREN

Comparative Enzyme Activity in Blood Serum and Effusion (in German). U. GERLACH* and H. KRONSBEIN. *Klin Wschr*, June 1, 1959, 37: 595–597. (* Wilhelms-Universität, Münster i. Westf., Germany)

The activity of lactic acid dehydrogenase was greater in the effusions (mostly pleural) than in the serum of 32 patients. This activity is about as great in effusions developed as a consequence of an inflammatory process as in those associated with malignancy. The noninflammatory effusions—connected with congestive heart failure, cirrhosis of the liver, or chronic nephritis—showed lesser enzyme activity than did the serum of the same patient. The determination of enzyme activity is not a fool-proof method for differentiation between malignant and inflammatory effusions. —Z. VIRÁGH

Chronic Idiopathic Pericardial Effusion: With Special Reference to the Development of Constrictive Pericarditis. J. SCHEUER*. *Circulation*, January, 1960, 21: 41–48. (* Mount Sinai Hosp., New York, N. Y.)

Chronic idiopathic pericardial effusion is an unusual condition that causes severe congestive heart failure and may be an early stage in the development of constrictive pericarditis. The condition must be recognized early in its course before severe hepatic damage or constrictive pericarditis occur. Because the course cannot be predicted and because surgery provides maximal therapeutic result when performed in the early stages, pericardiectomy should be performed when the condition is discovered. A case of chronic idiopathic pericardial effusion in a middle-aged hypertensive woman is presented; the findings and courses of 18 similar cases from the literature are reviewed. —W. J. STEININGER

Treatment of Pleural and Peritoneal Effusion with Intracavitary Colloidal Radiogold (Au^{198}). J. DYBICKI*, O. BALCHUM, and G. MENEELY. *AMA Arch Intern Med*, November, 1959, 104: 802–815. (* Vanderbilt U. School of Med., Nashville, Tenn.)

Intracavitary radiogold was administered to 45 patients with pleural and peritoneal effusions due to local or metastatic malignancy; 39 of these patients could be completely evaluated. In 22 patients with pleural effusion, good results were obtained in 54.5 per cent, fair results in an additional 18.2 per cent, and failure resulted in the remaining 27.3 per cent. Compilation from the literature of 1,899 instances of radiogold administration to patients with malignant pleural and peritoneal effusions has indicated that good results have been achieved in 50 per cent (after authors' summary). —T. H. NOEHREN

Diethylcarbamazine in Tropical Pulmonary Eosinophilia. A. SHANKER*, R. K. BHARGAVA, and B. N. SHRIVASTAVA. *Brit Med J*, January 9, 1960, No. 5166: 100–104. (* Ghandi Med. College, Bhopal, India)

Fifty patients with tropical pulmonary eosinophilia were treated with diethylcarbamazine. The drug was administered in a daily dose of 8 mg. per kg. of bodyweight. A cure rate of 98 per cent was obtained. There were no toxic reactions. Parenteral therapy was disappointing. Eighty-six per cent of the patients were cured after 16 days of treatment. The results obtained are superior to those after arsenic therapy.—E. A. RILEY

Diethylcarbamazine Therapy in Tropical Eosinophilia. K. NATH* and S. N. PANDEYA. *Brit Med J*, January 9, 1960, No. 5166: 104–107. (*Lucknow U., India)

Among the cases of tropical eosinophilia treated by diethylcarbamazine, 40 (91 per cent) were cured in 2 weeks. There were no side reactions. The drug is superior to arsenic.—E. A. RILEY

Pulmonary Symptoms and Eosinophilia Due to Filariasis. R A. RAICH* and D. F. GLEASON. *Tubercle*, December, 1959, 40: 462–465. (*V.A. Hosp., Minneapolis, Minn.)

Asthma, transient opacities of the lungs, adenopathy, and eosinophilia may be associated with filariasis. This helminthic infection of lymphatic vessels and lymph nodes consists typically of benign lymphadenitis and lymphangitis, with infrequent lymphatic obstruction, and consequent "lymph scrotum" or elephantiasis. Pulmonary symptoms are uncommon.

A 28-year-old farmer entered the hospital in September, 1958, complaining of severe progressive shortness of breath, wheezing, and cough for 2 days. Attacks of lesser severity had been occurring since 1954, when the patient first experienced asthma when returning from 13 months of military duty in South Korea. Alcoholic beverages caused a feeling of tightness and pain in the anterior chest and left arm. In 1957, a firm, painless mass was noted in the left axilla. The patient had lived his entire life in northern Minnesota, U.S.A., except for the military duty in Korea, with short stays in Japan and the Hawaiian islands. The chest roentgenogram showed linear markings in the lower zones consistent with focal atelectasis. The leukocyte count was 17,700 per cu. mm., with 74 per cent eosinophils. The mass in the left axilla was excised and showed a filarial worm, probably *Wuchereria malayi*, and the patient was treated successfully with diethylcarbamazine. Six months later the leukocyte count was 5,350 per cu. mm., of which 9 per cent were eosinophils. Ten months later he still had no recurrence of asthma.

Filariasis has been implicated as an etiologic factor in tropical eosinophilia, also called the eosinophilic lung. However, this unexplained eosinophilia with pulmonary symptoms and transient pulmonary opacities rarely occurs with significant adenopathy. Alcohol intolerance, heretofore not reported with this syndrome, is probably not allergic, but may be associated with the lymphoid hyperplasia; it has been reported in macrofollicular lymphoma and other conditions, and may not be specific for Hodgkin's disease. —M. J. Small

Honeycomb Lungs with Pericholangitis. S. Sivanesan*. *Arch Dis Child*, October, 1959, 34: 426–430. (*U. of Malaya, Singapore)

A case of honeycomb lungs associated with liver lesions is presented. In this case and in the 3 cases previously reported, the disease occurred in children between 2 and 3½ years of age, with an onset of dyspnea, cough, bizarre findings in the lungs on auscultation, and enlarged liver. At autopsy, both lungs showed honeycombing and there was an enlarged liver with pericholangitis. The nature of the lesion is discussed. It is concluded that in the pathogenesis both congenital and acquired factors are involved (Author's summary).—K. Deuschle

Configuration of Elastic Tissue of Pulmonary Trunk in Idiopathic Pulmonary Hypertension. D. Heath* and J. E. Edwards. *Circulation*, January, 1960, 21: 59–62. (*Mayo Clinic, Rochester, Minn.)

Study was made of the configuration of the elastic tissue of the pulmonary trunk in 6 cases of idiopathic pulmonary hypertension. The ages of the patients ranged from 12 to 54 years. The pattern was that of the adult pulmonary type in the 5 female patients, whereas it more closely resembled the fetal, aorta-like type in the single male patient. These data are interpreted as indicating that idiopathic pulmonary hypertension in most cases is acquired, but that it may be present from birth in a minority of instances. —W. J. Steininger

The Mediastinal Syndrome (in Spanish). E. Escobar Cerda* and H. Ruiz Pulido. *Rev Chile Enferm Torax*, No. 2, 1958, 23: 98–115. (*Hosp. San Francisco de Borja, Santiago, Chile)

This disorder is the result of compression of 1 or more organs located in the mediastinum. The symptoms vary according to the organ affected; they can be the first manifestations of a mediastinal malady. In the decade 1948–58, there were 197 patients admitted to the Hospital San Borja in Santiago, Chile, 18 of whom developed the syndrome. The breakdown: 8 patients of 15 with aneurysm of the aorta, 8 of 43 with bronchogenic carcinoma, and 2 patients of 15 with lymphoma (among the lymphomas, there were 6 patients with Hodgkin's disease, 7 with lymphosarcomas, and 1 reticulosarcoma). None of the 118 patients with esophageal carcinoma nor the 6 with chronic lymphatic leukemia presented symptoms referable to the compression syndrome. The diagnostic problem represented by the mediastinal syndrome should be solved by exploratory thoracotomy. —Z. Virágh

Melioidosis (in Turkish). C. Ertug*. *Tuberk Toraks*, September–December, 1959, 7: 31–41. (*U. of Ankara, Turkey)

Melioidosis is an infectious disease caused by *Bacillus whitmori*, or *Malleomyces pseudomallei*. The organism usually produces the clinical picture of an acute generalized septicemia with multiple and diffuse abscesses involving almost every organ of the body. The chronic type of this disease, which resembles glanders, is rare. About 500 cases have been reported to date, all from Far-eastern countries. The bacteriologically proved case of pulmonary melioidosis which is reported in this paper is the first ever to have been reported in the Western hemisphere.—S. Solu

Ornithosis: A Case With Miliary Densities of Both Lung Fields and an Exceptional Course (in Norwegian). J. O. Alvsaker*. *Nord Med*, October 22, 1959, 62: 1550–1552. (*Fylkessjukehuset, Ålesund, Norway)

A case is reported of pulmonary ornithosis with symmetric miliary densities of both lung fields,

and a rather protracted course complicated by changes in the heart contour and reduced vital capacity. There was no evidence of primary heart involvement. Corticoid therapy produced striking improvement. It was suggested that this case might represent an intermediate stage between a benign viral infection and the Hamman-Rich syndrome.—J. HAAPANEN

Late Progression of Pulmonary Coccidioidomycosis. M. J. SMALL*. *AMA Arch Intern Med*, November, 1959, 104: 730–739. (*V.A. Hosp., East Orange, N. J.)

Four cases have been observed within a relatively short space of time which strongly tend to indicate that pulmonary coccidioidomycosis, contrary to the commonly held current concepts, may be a disease which: (*1*) does not manifest itself until a prolonged period after exposure in the endemic area; (*2*) may undergo late, continued, slow progression over a period of years; and (*3*) may spread by the bronchial route from cavities previously considered benign to produce infiltrations years later in the lung, indistinguishable by roentgenogram from those of exudative tuberculosis. Since all of these cases were observed in persons residing in the eastern part of the country, whose roentgen manifestation of disease did not become manifest until 5 months to many years after their documented residence in a known endemic area for coccidioidomycosis, it would appear that late pulmonary manifestations of coccidioidomycosis must be added to the list of differential diagnoses when considering stationary or progressive coin lesions of the lung, cavitary lesions without positive bacteriology for tuberculosis, and parenchymal infiltrations resembling tuberculosis, even years after exposure in an endemic area (after author's summary).—T. H. NOEHREN

Disseminated Cryptococcosis Producing a Sarcoid Type Reaction. L. SHIELDS*. *AMA Arch Intern Med*, November, 1959, 104: 763–769. (Harrisburg Polyclinic Hosp., Penn.)

A report is made of a case of disseminated cryptococcosis which presented with symptoms of acute polyarthritis. The diagnosis of cryptococcosis was indisputably established. Many of the aspects of the disease pattern, however, suggested sarcoidosis. The case is unusual in that the infective cryptococci were not only associated with sarcoidosis, but were actually demonstrated in the area of sarcoid-type response, supporting the concept that the sarcoid reaction may be caused by various etiologic agents. Clinical remission was induced by the use of intravenous amphotericin B, and the acute and chronic toxicities of the drug are discussed (after author's summary).—T. H. NOEHREN

The Protean Manifestations of Histoplasmosis as Illustrated in Twenty-Three Cases. F. CONRAD*, S. SASLAW, and R. ATWELL. *AMA Arch Intern Med*, November, 1959, 104: 692–707. (*Ohio State U., Columbus)

Twenty-three cases of histoplasmosis are presented with emphasis on their symptomatology and mode of diagnosis. Seven patients had acute disease resembling common respiratory infections: 1 died after dissemination of the disease, 1 required a lobectomy, and the others cleared spontaneously with only symptomatic care. Eight patients had chronic, debilitating pulmonary disease resembling tuberculosis. Five of these patients were treated for tuberculosis, and 3 actually had concurrent or past cultures positive for *M. tuberculosis*. Four of the patients with chronic histoplasmosis exhibited exacerbations and remissions during the course of their illness. One patient presented with multiple arthralgias and weakness. He was thought to have sarcoidosis, but was shown to have a disseminated histoplasmosis. He has cleared completely on a regimen of modified rest alone. One patient was diagnosed to have a classical hypersplenism. She died with cerebral vascular hemorrhage secondary to a thrombocytopenia (after authors' summary).
—T. H. NOEHREN

Treatment of Childhood Pneumonia with a Single Dose of Sulfonamide (in German). H. EGGER* and J. SIEGL. *Wien Med Wschr*, May 2, 1959, 109: 393–394. (*Mautner-Markhofsches Kinderspital, Wien, Austria)

All but 7 of 104 children with pneumonia became afebrile and showed clinical improvement on the first day after administration of a single large dose of sulfonamide. In a period of 3–4 days, all but 10 per cent of the children reacted favorably. No side reactions or complications were observed. The amount of sulfonamide to be given is determined by the child's age and body weight.—Z. VIRÁGH

Primary Chronic Pneumonia (in German). W. BRUNNER* and E. TANNER. *Schweiz Z Tuberk*, No. 3–4, 1959, 16: 142–155. (*Bergstrasse 48, Zurich, Switzerland)

This term is applied to an inflammatory process of the lungs which does not result in resorption after 6 weeks. Primary chronic pneumonia is the sequel and result of an unresolved acute pneumonia or (rarely) it may develop from a chronic

interstitial inflammatory process. The anamnesis reveals an acute pneumonia-like beginning, but there are few symptoms. Histology shows organization of fibrinous exudate and cell-rich connective tissue in the alveoli or in the interstitium. Metaplasia of the bronchial epithelium may ensue, making a tumor cell examination of the bronchial secretion difficult. Circumscript residuals of an extensive infiltration, Wassermann-positive infiltrations, fungous diseases, Freidländer and pneumococcus (type III) pneumonias do not belong to this group. Similarly, the pneumonic processes associated with bronchiectasis, bronchial stenosis, or pulmonary infarction are also excluded as they are secondary chronic pneumonias.

Tuberculosis and the neoplasms should be considered in the differential diagnosis. Chronic pneumonia is discernible from tuberculosis by the greatly increased sedimentation rate of the erythrocytes, the enlargement of the regional lymph nodes, the absence of tubercle bacilli in a cavitary lesion revealed by planigrams, severe chest pain, and by the unusually dense bronchopneumonial shadow on the roentgenograms. Points of evidence against a diagnosis of tumor are: free passage in the bronchi experienced by bronchoscopy and, mainly, by bronchography; the clear, serous pleural fluid, when effusion is present; and the lack of tumor cells in bronchial secretions. In certain cases, however, it was necessary to resort to pulmonary resection.

Of 8 patients (6 men and 2 women), 5 were treated with penicillin and 1 with sulfonamides. Two male patients were thought to have tuberculosis and were treated symptomatically. Four patients underwent pulmonary resection for suspected bronchogenic carcinoma; in these cases the diagnosis of chronic pneumonia was established by histology of the resected specimen. Complete or partial resorption of chronic primary pneumonia is possible (it occurred in 3 of the presented cases); scarring and shrinking of the lesion are frequent.—Z. VIRÁGH

Generalized Cytomegalic Inclusion-Body Disease Associated with Pneumocystis Pneumonia in Adults. W. ST. C. SYMMERS*. *J Clin Path*, January, 1960, 13: 1–21. (*Charing Cross Hosp., London, England)

A report is presented of 3 patients with Wegener's granulomatosis, thrombotic purpura, and Hodgkin's disease as predisposing conditions. In all 3 cases it seems likely that the underlying disease, or the drugs used in its treatment, predisposed to cytomegalic inclusion-body disease and concomitant pneumocystis pneumonia by lowering the patient's resistance.—E. DUNNER

The Demonstration of Intranuclear Inclusion Bodies in Sputum from a Patient with Varicella Pneumonia. B. WILLIAMS* and T. CAPERS. *Amer J Med*, November, 1959, 27: 836–839. (*U. of Texas, Austin)

The diagnosis of varicella pneumonia has been made by a combination of clinical signs and symptoms together with the findings on the chest roentgenogram. Some radiologists claim that the diagnosis can be made from the clinical information and roentgenographic findings, which are frequently described as a bilateral, diffuse, mottled, miliary infiltration. However, some 80 different disease entities have been reported to be capable of producing this roentgenographic appearance. There is, then, a definite need to establish a positive etiologic diagnosis of the type of pneumonia when it develops in association with varicella. An adequate solution to this problem would be a positive pulmonary cytologic diagnosis. A review of the literature failed to reveal any positive cytologic diagnosis of varicella pneumonia except from post-mortem pathologic examination. This case is presented because the "Type A intranuclear inclusion bodies" pathognomonic of varicella were successfully recovered and identified from the sputum cells of an adult with varicella pneumonia.—T. H. NOEHREN

The Pathology of Psittacosis. A Report of Two Cases With Hepatitis. E. YOW*, J. BRENNAN, J. PRESTON, and S. LEVY. *Amer J Med*, November, 1959, 27: 739–749. (*Jefferson Davis Hosp., Houston, Texas)

Psittacosis in humans is a disease of increasing importance. It occurs not only sporadically following exposure to psittacine birds, but epidemics are being encountered among poultry workers exposed to chickens, ducks, and turkeys. Psittacosis varies in severity from a mild, relatively asymptomatic, influenza-like illness to a severe systemic, fatal disease. The clinical features of turkey-borne psittacosis are described as they were observed in an outbreak infecting 24 of 40 workers.

In human beings, the lung is the organ most consistently involved, although occasional patients exhibit no clinical or roentgenographic evidence of pneumonia. As is true of the other viral forms of pneumonia, the usual physical findings are not obvious during the first few days of fever. Even changes in the pneumonia as seen in the roentgenograms may not appear until later in the course of the illness. The sputum is quite scanty, but increases in severe illness and during the stage of resolution. It is nonpurulent and mucoid in character. Of particular clinical interest is the relatively inconspicuous cough, in contrast to the

severe cough so characteristic of most other forms of viral pneumonia. While some patients complain of pain or soreness in the chest, true pleuritis is quite rare. This is surprising in view of the polyserositis of the disease in birds. Splenomegaly is sometimes noted for a few days toward the end of the first week of illness. This is more common in those patients with serious illnesses. Signs and symptoms of pulmonary insufficiency are seen only in the most severe cases.—T. H. NOEHREN

Auto-immune Thrombocytopenic Purpura as a Complication of Pulmonary Hydatid Cyst in a Four-Year-Old Child (in Turkish). F. TEZOK*, F. SERTER, and K. KURTAR. *Turberk Toraks*, September-December, 1959, 7: 104–116. (*Military Medical Academy, Ankara, Turkey)

A case of thrombocytopenic purpura due to hypersensitivity to pulmonary *Echinococcus granulosus* is described. The role of hydatid cyst liquid in this auto-immunology and the relationship between thrombocytopenic purpura and *Echinococcus granulosus* are discussed. The importance is emphasized of the use of hydatid cyst liquid obtained from human sources instead of animals for the Casoni skin test.—S. SOLU

Sarcoidosis and Hyperparathyroidism with Hypercalcemia: Special Usefulness of the Cortisone Test. J. M. BURR*, J. J. FARRELL, and A. G. HILLS. *New Engl J Med*, December 17, 1959, 261: 1271–1275. (*U. of Miami School of Med., Florida)

A third case is reported of coexisting sarcoidosis and hyperparathyroidism with hypercalcemia. The cortisone test was of particular value in establishing that the hypercalcemia was due to hyperparathyroidism. Because of the coexistence of hypercalcemia and its sequelae—hyperglobulinemia and hepatosplenomegaly—in a Negro patient, sarcoidosis was the favored diagnosis during the initial portion of the patient's admission to the hospital. However, a history of nephrolithiasis dating back 8 years, hypertension, low serum phosphorus concentrations and, especially, an abnormally low value for the phosphorus reabsorption index, necessarily raised the question of possible hyperparathyroidism. The failure of administered cortisone to affect the hypercalcemia and hypercalciuria was considered to exclude sarcoidosis as their cause, and led to the presumptive preoperative diagnosis of hyperparathyroidism. Surgical removal of a parathyroid adenoma, followed by prompt relief of hypercalcemia and hypercalciuria, confirmed the diagnosis of hyperparathyroidism and showed that the hypercalcemia had been ascribable to hyperparathyroidism. The diagnosis of sarcoidosis was established when histologic evidence of the disease was obtained in the resected spleen and abdominal lymph nodes. On the basis of available information, it appears that a typical positive response to the cortisone test excludes hyperparathyroidism as a cause of the hypercalcemia, whereas failure to respond is consistent with, though by no means diagnostic of, hyperparathyroidism.—M. J. SMALL

Sudden Death Due to Myocardial Sarcoidosis, with a Comment on the Etiology of Sarcoid. T. POON* and W. FORBUS. *AMA Arch Intern Med*, November, 1959, 104: 771–777. (*Duke U. School of Med., Durham, N. C.)

Two cases of sudden death due to widespread myocardial sarcoid granuloma are described, bringing the total on record to 17 (Authors' summary).—T. H. NOEHREN

Suxamethonium Apnoea in an Infant. L. KAUFMANN*, H. LEHMANN, and E. SILK. *Brit Med J*, January 16, 1960, No. 5167: 166–167. (*Hosp. for Sick Children, London, England)

Succinylcholine (suxamethonium) apnea is described in a child aged 15 months. This is believed to be the first such recorded observation in an infant. The family has been studied in detail. There was a deficiency of pseudocholinesterase in 1 maternal and 1 paternal grandparent, yet only low normal values for pseudocholinesterase were found in both parents. The overlap of enzyme levels found for presumed heterozygotes for the abnormal pseudocholinesterase gene and for normal individuals is discussed (after authors' summary). — E. A. RILEY

Pulmonary Aspergilloma (in German). A. FINGERLAND*, O. SKŘIVÁNEK, F. MYDLIL, and J. PROCHÁZKA. *Z Tuberk*, September, 1959, 113: 284–303. (*Pathologisch-Anatomisches Institut, Hrádec Králové, Czechoslovakia)

Eight cases of aspergilloma are described. The characteristic feature is the mycelium formation in a pre-formed cavity without conidia. The preformed cavities in this material were: healing tuberculous cavities in 2 instances, bronchiectasis in 5, and chronic lung abscess once.—J. HAAPANEN

Esophageal Cysts. G. DESFORGES* and J. W. STRIEDER. *New Engl J Med*, January 14, 1960, 262: 60–64. (*Tufts U. School of Med., Boston, Mass.)

Esophageal cysts form a group of rare and interesting mediastinal tumors. Three cases are pre-

sented. The first presented as a hilar mass, and was discovered incidental to exploratory thoracotomy for a preoperative diagnosis of lung tumor. The second and third showed striking symptomatology in the form of dysphagia and hemothorax, respectively. The embryologic theories of development of this type of cyst are reviewed. In view of the likelihood that esophageal cysts arise as a result of accessory buds pinched off from the primitive foregut, "primitive foregut cyst" is more accurate terminology. Since these cysts are potentially symptomatic, surgical excision should be considered whenever one is discovered.
—M. J. SMALL

Esophagogenic Mediastinal Cyst (in Finnish). E. MERIKALLIO*. *Duodecim*, December, 1959, 75: 927–931. (*Kirurginen Klinikka, Helsinki, Finland)

A 46-year-old female had complained of epigastric pain for years. The pain radiated to the neck and shoulders. Dysphagia was present and the patient became short of breath after even the slightest degree of exertion. Roentgenographically, a large smooth tumor was diagnosed in the lower mediastinum. After surgical removal, microscopic examination showed esophagogenic tissue with secreting glands. The patient had a successful recovery after the operation.—J. HAAPANEN

Malignant Pleural Mesothelioma (in Spanish). M. L. SELDON* and J. L. REY. *Enferm Torax*, No. 32, 1959, 8: 379–409. (*Sanatorio Antituberculoso de Compostela, Spain)

A 24-year-old farmer was found to have a large homogenous, oval-shaped dense mass in the upper paramediastinal area. His only complaint was some chest pain on the right. The patient died 7 days after surgical removal of the tumor, which was diagnosed histopathologically as a malignant mesothelioma. Another patient, a 75-year-old farmer, was admitted to the hospital with complaints of fatigue, constant cough, ample expectoration, anorexia, and general weakness of about 3 months' standing. Roentgenograms revealed innumerable small nodular densities disseminated in the lungs, and more pronounced in the perihilar regions. Many small nodes could be found subcutaneously over the chest; some of these were necrotized and, when biopsied, the diagnosis of "pulmonary malignant neoplasm with subcutaneous metastases" was established. Further studies revealed that these nodes were epithelial type mesotheliomas. This disorder is sometimes called pleuroma. It is subdivided histologically into epithelial and fibrous types. Both of these varieties might be malignant or benign. Skin metastasis is rare.—Z. VIRÁGH

Pulmonary Myomatosis (in Hungarian). P. KESZLER*, GY. SZINAY, and L. VOITH. *Tuberkulozis*, June, 1959, 6: 131–134. (*U. of Budapest, Hungary)

The case history is presented of a 34-year-old male patient who was admitted to hospital as a diagnostic problem; the objective finding of stenosis of the left main bronchus was revealed by bronchoscopy and bronchography. Pneumonectomy was performed and the left lower lobe lung parenchyma was found to be completely replaced by muscle tissue. The pathologic diagnosis was myomatosis, probably hamartomatosis.
—Z. VIRÁGH

Nonchromaffin Paraganglioma (Chemodectoma) of the Mediastinum (in German). G.-H. CHIU* and J. B. CARVALHO. *Z Tuberk*, September, 1959, 113: 330–336. (*Chirurgische Klinik, Medizinische Akademie, Düsseldorf, Germany)

A case is reported of a nonchromaffin paraganglioma (chemodectoma) of the mediastinum. This appears to be the tenth case ever reported, and the first from Germany. The patient was 27 years old. An attempt was made to remove the tumor surgically but, because of its diffuse character and adhesion to the blood vessels, a radical removal was not possible and the operation resulted in an exploratory thoracotomy.
—J. HAAPANEN

Malignant Tumors Associated with Dermatomyositis (in German). R. HEGGLIN* and W. SIEGENTHALER. *Schweiz Z Tuberk*, No. 3–4, 1959, 16: 205–221. (*Medizinische Universitäts-Poliklinik, Ramistrasse 100, Zurich, Switzerland)

A 49-year-old female patient was treated with prednisone for dermatomyositis (confirmed histopathologically). After about 18 months of steroid administration, generalized tuberculosis (miliary tuberculosis, meningitis, cold abscess) developed and the prednisone therapy had to be discontinued. Intensive antituberculosis treatment followed and the patient's condition became quite satisfactory, until symptoms presented indicating new pulmonary and mediastinal processes. Roentgenograms and histologic examination of the specimen obtained by biopsy of a tumor-like mass in the left chest revealed malignancy. About 4 months later, the autopsy ascertained the diagnosis of metastatic thymic carcinoma. Malignant neoplasms develop in 18 per cent of cases of dermatomyositis.—Z. VIRÁGH

Primary Heart Tumors (in Norwegian). K. MAURSETH*. *Nord Med*, October 1, 1959, 62: 1452–1455. (*Rikshospitalet, Oslo, Norway)

Two cases of primary heart tumors are described: a fibrochondroangiosarcoma of the left atrium, and a cavernous hemangioma on the surface of the right ventricle.—J. HAAPANEN

Role of PAP (Eaton-Liu) Virus in Childhood Respiratory Disease. M. K. COOK*, R. M. CHANOCK, R. H. PARROTT, and R. J. HUEBNER. *AMA J Dis Child*, November, 1959, 98: 575–576. (*National Institute of Allergy, Bethesda, Md.)

The indirect fluorescent-antibody technique was employed to determine if PAP (Eaton-Liu) virus or an agent antigenically related to it plays a role in childhood respiratory disease. One hundred and ten infants and children with undiagnosed lower respiratory tract illness were studied for evidence of PAP infection. The findings suggest that PAP virus or an agent (or agents) antigenically related to it was associated with 16 per cent of the undiagnosed lower respiratory illness in Washington during 1957–1959. Since 60 per cent of the cases of lower respiratory illness were etiologically undiagnosed, an estimate of the contribution of PAP virus to lower respiratory tract disease would be 10 per cent.—M. J. SMALL

TUBERCULOSIS, PULMONARY

Repeated Isolation of Atypical (Unclassified) Acid-fast Bacilli from a Case of Pulmonary Tuberculosis (in Japanese). Z. NAKAMURA*, M. SUGIUCHI, K. HIBI, and M. AOKI. *Jap J Chest Dis*, February, 1960, 19: 134–139. (*Nihon Kokan Kiyose Yokufuen, Tokyo, Japan)

The writers report a 23-year-old male with pulmonary tuberculosis from whom unclassified acid-fast bacilli were repeatedly isolated by culture from the sputum and by smear and culture from the resected specimen. The bacilli formed yellow colonies which were: scotochromogenic (yellow colonies developed both in the dark and in the light, but they gradually became orange when cultured in the light); catalase positive; neutral red- and sodium sulfide-fuchsin negative; they lacked cord factors; they grew well on medium containing 0.5 per cent lead acetate, but not on medium containing 0.05 per cent potassium tellurite and potassium tellurate. They were resistant to most of the antituberculosis drugs. The pathologic anatomy of the resected lung specimen (segment 1 of the right upper lobe) which contained a cavity was quite similar to the usual forms of pulmonary tuberculosis. The therapeutic complications of this type of pulmonary tuberculosis are discussed.—I. TATENO

Blood Serum Proteins in Tuberculous Patients (in Russian). V. I. GRIGORIEVA* and N. A. LIVSHITZ. *Lab Delo*, January–February, 1960, 1: 8–11. (*Pavlov's Leningrad Med. Institute, U.S.S.R.)

In order to establish the influence of duration and extent of tuberculosis upon the metabolism of proteins, determinations of total proteins, albumin, and globulin fractions were made in the blood serum of 79 tuberculous patients and 10 controls. The rise in beta globulin was proportional to the severity and extent of disease and to the degree of amyloidosis. High cholesterol and high beta-globulin readings were found in a group of 17 patients; however, no direct relationship between the two components could be demonstrated. There was no definite pattern of relationship between the character of disease and the gamma globulins. Increase in occurrence of amyloidosis could not be attributed to longer life-span of tuberculous patients. Necessity for an adequate protein diet is stressed. Antibacterial therapy does not prevent the development of abnormal protein metabolism.—A. V. POPOFF

Pseudocavitary Area of the Lung during Treatment with Pneumothorax (in Italian). L. AGAZIA* and A. MACCHI. *G Ital Tuberc*, November–December, 1959, 13: 385–392. (*Osp. Sanatoriale, Venice, Italy)

Four cases are described of patients treated with pneumothorax who had to be hospitalized again because of the appearance of a transparent area in the lung which was thought to be a cavity. Following complete re-expansion of the lung, given favorable clinical and laboratory data, the pseudocavitary area disappeared. The main criteria in identifying these formations were: (*a*) the lack of correspondence between the area and the site of the initial pulmonary lesion; (*b*) the contrast between the roentgenographic aspect and the lack of any symptoms of an active tuberculous process; and (*c*) the normal values of biologic tests such as the blood sedimentation rate, the electrophoretic pattern of plasma proteins, et cetera, and the constant lack of tubercle bacilli in the sputum.—I. ARCHETTI

Treatment of Active Tuberculosis in Silicotic Patients (in Italian). E. C. VIGLIANI* and C. SASSI. *Med Lavoro*, October, 1959, 50: 593–597. (*U. of Milan, Italy)

Eighty-five patients with silicosis associated with active forms of tuberculosis were treated from 1955 to 1958 for a period varying from 2 to 5 months, using the following different drug combinations: (*1*) 2 gm. of streptomycin on the first day, 12 gm. of PAS on the second, and 300 mg. of

isoniazid on the third; (2) 450 mg. of isoniazid daily and 2 gm. of streptomycin three times a week; (3) 700 mg. of isoniazid combined with 22 gm. of PAS daily, given by intravenous perfusion; (4) 400 mg. of isoniazid combined with 0.5 to 0.75 gm. of cycloserine daily. Forty-five patients showed clear improvement, 35 remained stationary, and 5 worsened. The results obtained using the various combinations were about the same, except for the isoniazid–streptomycin regimen, which gave less favorable results.
—I. Archetti

The Pathogenesis of Tuberculoma (in German). F. W. Wolf*. *Beitr Klin Tuberk*, May, 1959, 120: 170–185. (*Bremen, Heinrich-Hertz-Str. 38, Germany)

A study of the case histories of 210 patients with tuberculomas revealed that tuberculoma formation is possible in any phase of the process. It develops mostly in the age between puberty and the climacteric, reflecting the effects of stress, metabolic disease, and gravidity on the body.
—Z. Virágh

TUBERCULOSIS, NONPULMONARY

Errors in Diagnosis of Tuberculous Lymphadenitis (in Roumanian). N. Georgescu*, C. Tavernier, and Gh. Radulescu. *Ftiziologia*, March-April, 1959, No. 2: 171–178. (*Sanatoriu de Floresti, Roumania)

In the last 2 years, 117 patients were treated for tuberculous lymphadenitis at Floresti. The diagnosis was erroneous in about 10 per cent of the cases. The case histories are presented of 5 patients; in 4 the diagnosis (1 Hodgkin's disease, 1 benign adenopathy, 2 neoplasms) could be established by needle biopsy of a lymph node. The fifth patient's diagnosis of reticulosarcoma was made by histologic examination of a surgically removed lymph node. Needle biopsy is a simple procedure and should be performed in every patient with lymphadenopathy.—Z. Virágh

Ophthalmic Involvement in Tuberculous Meningoencephalitis (in Roumanian). F. Lascu* and M. Niculescu. *Ftiziologia*, March-April, 1959, No. 2: 179–186. (*Sectia de Ftizio-Pediatrie a Institutului de Ftiziologie, Bucuresti, Roumania)

Of 172 patients with tuberculous meningitis, the eyes of 124 were also affected (72.1 per cent). Tuberculous choroiditis was diagnosed in 5 patients, papilledema in 48, and optic nerve involvement in 69 (mostly primary atrophy). Two patients developed hemianopsia. The prognosis of these ocular manifestations depends on the intensity of the treatment (by antimicrobials and steroids).—Z. Virágh

Sugar Content Changes in the Spinal Fluid of ACTH (Corticotropin)-treated Tuberculous Meningoencephalitis of Children (in German). T. Loós* and M. Lőrinczy. *Beitr Klin Tuberk*, May, 1959, 120: 186–194. (*Vármegyei Közkórház, Nyiregyháza, Hungary)

Corticotropin is usually employed to prevent the development of obstructive hydrocephalus in children with tuberculous meningoencephalitis. The beneficial effect of the hormone is attributed to its ability to dissolve the thick fibrinous exudate on the basal meninges, preventing the development of endarteritis, and increased permeability of the vessels. These effects are thought to allow permeation of tuberculous foci by the antimicrobials. This would explain the fact that the sugar content of the spinal fluid usually reaches the normal of 50–60 mg. per 100 ml. from a low of 20 mg. per 100 ml. after 5–6 days of hormone treatment. When corticotropin is discontinued, the values go back to the original low.

The permeability of the blood vessels of the brain was tested by intravenous administration of 40 per cent dextrose to children with meningoencephalitis during treatment with corticotropin. In all of these experiments, not only the spinal fluid sugar content but the blood sugar content as well became elevated; therefore, it is suggested that corticotropin does not possess a special faculty directed at increasing the permeability of the blood vessels of the brain.—Z. Virágh

Tuberculosis of the Cervical Lymph Nodes (in German). V. Kesztele*. *Wien Med Wschr*, May 23, 1959, 109: 433–435. (*Allgemeines Krankenhaus, Linz, Austria)

In 70–90 per cent of cases, cervical lymph node tuberculosis is caused by bovine tubercle bacilli. An investigation in upper Austria revealed that 19.2 per cent of the cattle were infected by tuberculosis. At the present time, prevention of tuberculous infection from dairy products is not satisfactory. In the last 3 years, 67 patients of all ages and both sexes were admitted to hospital with cervical lymphadenitis. Of these, 2 cases were due to ascariasis, 3 to mononucleosis, and 62 to tuberculosis. Treatment included the antimicrobials—streptomycin, isoniazid, and PAS—as well as surgery (41 patients). The results were very gratifying; among those operated on, there were 4 recidivations. The importance of prevention is emphasized.—Z. Virágh

Complete Lack of Resistance to Tuberculosis in Childhood (in German). J. G. DETMOLD*, G. JOPPICH, and D. SINAPIUS. *Deutsch Med Wschr*, January 15, 1960, 85: 104–110. (*Kinderklinik, Göttingen, Germany)

A 2½-year-old child contracted miliary tuberculosis, which recurred twice within the following 3 years. Human and bovine strains of *M. tuberculosis* were found in large amounts in the gastric contents, urine, and cerebrospinal fluid. For a time there was also a tuberculous ulcer in the vagina. Tuberculin sensitivity developed for only a short period and was never of high degree. Bacterial allergy was absent. There was no agammaglobulinemia. The child died of tuberculous meningitis. The post-mortem histologic examination revealed a nonspecific inflammatory reaction; there were no tubercles or any caseation. No primary complex was found. The most marked morphologic changes were found in the ventricular system (tuberculous ependymitis).—J. HAAPANEN

UPPER RESPIRATORY TRACT DISEASE, VIRAL

The Contribution of Detectable Viruses to the Croup Syndrome. R. H. PARROTT*, R. M. CHANOCK, and A. J. VARGOSKO. *AMA J Dis Child*, November, 1959, 98: 626. (*Children's Hosp., Washington, D. C.)

Of 47 children with infectious croup who were studied during a 1-year period, a diagnosis of virus could be made in 64 per cent. Type 2 hemadsorption virus infection was found in 36 per cent of all croup patients and was associated with illness throughout the year. Asian influenza virus alone was associated with 36 per cent of croup patients studied during the Asian influenza epidemic, and 19 per cent through the year. Nine per cent of croup illness was associated with adenovirus, Type 1 hemadsorption virus, or croup-associated virus. Type 2 hemadsorption virus infection was found predominantly in boys, but its clinical features were not distinctive (after authors' summary).—M. J. SMALL

THORACIC SURGERY

Experience and Results in the Conservative and Surgical Treatment of Lung Abscess (in German). W. KÖLE* and G. RECKENZAUN. *Wien Med Wschr*, May 9, 1959, 109: 419–423. (*Chirurgische Universitätsklinik, Graz, Austria)

Since spontaneous healing is possible, conservative treatment is first recommended in cases of acute lung abscess. This includes bronchoscopy (suction, rinsing, introducing drugs), endobronchial instillation with the Metras catheter, transthoracic application of antimicrobials into the lesion, aerosol treatment in addition to broad-spectrum antimicrobials administered orally, and symptomatic treatment as needed. By employing conservative treatment, 60 per cent of the 64 patients in this study were cured or satisfactorily improved; 2 died (3.1 per cent).

Forty-two patients were treated surgically; all types of pulmonary surgery were utilized in 52 operations. The mortality was 17.3 per cent. —Z. VIRÁGH

Needle Biopsy in the Diagnosis of Pulmonary Tumour. Y. PERTTALA*. *Ann Chir Gynaec Fenn*, Fasc. 4, 1959, 48: 427–436. (*14 Perustie, Helsinki, Finland)

Needle biopsy of lung tumors was performed 75 times on 63 patients. A definite histologic diagnosis was obtained in 38 per cent of the cases of carcinoma. Including suspected carcinomas (3 cases) and positive tumor cells (12 cases), the total number of positive findings was 68 per cent. A negative result does not exclude the existence of carcinoma. Three major complications occurred: empyema, hydropneumothorax, and severe pneumonia, 1 case of each. Three cases of minor pneumothorax and 3 cases of fever for 1 to 4 days after the biopsy represent minor complications.—J. HAAPANEN

Open Lung Biopsy for Diffuse Pulmonary Disease. L. J. GRANT* and S. A. TRIVEDI. *Brit Med J*, January 2, 1960, No. 5165: 17–21. (*London Chest Hosp., England)

Open lung biopsy was carried out on 26 patients with diffuse pulmonary disease. There was no operative mortality. Postoperative complications were minimal. A specific histologic diagnosis was made in 24 cases. In 18 cases the preoperative clinical diagnosis was incorrect.—E. A. RILEY

Non-Penetrating Chest Injury Resulting in Isolated Rupture of the Ventricular Septum and Angina Pectoris. G. A. FERUGLIO*, A. BAYLEY, and W. F. GREENWOOD. *Canad Med Ass J*, January 30, 1960, 92: 261–265. (*U. of Toronto, Canada)

A case of rupture of the ventricular septum accompanied by the development of angina pectoris is described in a previously well 37-year-old miner who suffered a severe nonpenetrating chest injury. Only 21 similar cases have been reported. —E. A. RILEY

Chest Surgery without Antimicrobial Coverage (in German). U. BALDAMUS* and M.-L. GLAVE. *Tuberkulosearzt*, November, 1959, 13: 752–758. (*Heim-Tbk-Krankenhaus, Berlin-Buch, Germany)

Because "hospital staphylococci" are resistant to penicillin, routine postoperative penicillin was

abandoned. Later on, postoperative streptomycin also was discontinued. A group of 53 patients were operated on for pulmonary tuberculosis with streptomycin coverage, and another group of 51 patients without any antimicrobial coverage at all. The incidence of postoperative complications due to staphylococcal infection was considerably lower in the latter group.—J. HAAPANEN

Nonobstructive Pulmonary Atelectasis following Thoracotomy (in Japanese). S. IZAI* and T. UENO. *Jap J Thor Surg*, February, 1960, 13: 129–134. (*Hirosaki U. School of Med., Japan)

Postoperative pulmonary atelectasis due to bronchial obstruction with secretions or to accumulation of exudates and blood in the thoracic cavity is sometimes seen following thoracotomy. However, the writers reported 2 cases of postoperative atelectasis without any obstruction to the air passage from within or from the outside. The first case was a 25-year-old woman with a residual cavity in the right lung following thoracoplasty, and the second case was a 45-year-old woman with esophageal diverticulum. Massive atelectasis developed in both cases, after lobectomy in the first and after thoracotomy in the second case. No obstruction to the air passage was demonstrated by bronchography and bronchoscopy. It is concluded that the impairment of respiratory movement of the thoracic cage was due to the unusually severe pain of which the patients complained, and this might have been the cause of disturbed ventilation of the lung resulting in atelectasis.—I. TATENO

The Importance of Exploratory Thoracotomy in Undiagnosed Chest Processes (in German). A. BRUNNER*. *Schweiz Z Tuberk*, No. 3–4, 1959, 16: 134–141. (*Chirurgische Universitätsklinik, Zurich, Switzerland)

After 11 years standing, a seemingly benign tumor began to enlarge and in 5 weeks became so big that a pneumonectomy was done, and a spindle-cell sarcoma was diagnosed histopathologically. The patient died from recidivation of the disease 5 months after the operation. Another patient, a salesman, underwent a right upper lobectomy for an egg-sized, sharply delineated, round mass revealed by roentgenograms. The histopathologic diagnosis of the surgically removed specimen was silicotuberculosis. Tubercle bacilli were demonstrated. Only after the operation was the occupational history revealed, supporting the diagnosis of silicosis. The importance of exploratory thoracotomy in pulmonary diagnostic problems is evidenced by this report.
—Z. VIRÁGH

Mediastinal Fibroma. E. LAUSTELA*. *Ann Chir Gynaec Fenn*, Fasc. 4, 1959, 48: 505–517. (*Kirurgi, Helsinki, Finland)

Five cases of fibroma and 1 case of hemangiofibroma were diagnosed among 200 cases of mediastinal tumor between 1950 and 1958. All 6 cases are described in detail. Four were symptomless, detected by routine roentgenography. One patient had dyspnea and pain; another had chest pain. In all of the cases the tumors were removed surgically and recovery has been complete.—J. HAAPANEN

Selection for Surgery of Patients with Ventricular Septal Defect and Pulmonary Hypertension. J. W. DUSHANE* and J. W. KIRKLIN. *Circulation*, January, 1960, 21: 13–20. (*Mayo Clinic, Rochester, Minn.)

Estimation of pulmonary blood flow relative to systemic blood flow is a critical point in selecting patients with ventricular septal defect and severe pulmonary hypertension for operation. A detailed medical history, physical examination, thoracic roentgenograms, careful interpretation of electrocardiograms, cardiac catheterization, and biopsy of the lung provide approaches to the problem. In borderline cases, decision still may be extremely difficult. The authors usually favor surgery, however, with recognition that this decision may be in error. Obviously, proper evaluation for operation cannot assure uniformly good results. Method of conduct of operation, perfusion, and postoperative care determine the results.
—W. J. STEININGER

ROENTGENOGRAPHY

Radiological Diagnosis of Anomalous Pulmonary Venous Connection: A Tomographic Study. F. DALITH* and H. NEUFELD. *Radiology*, January, 1960, 74: 1–18. (*Government Hosp., Tel-Hashomer, Israel)

Anomalous pulmonary venous connection, which may be either partial or complete, is a congenital malformation in which one, several, or all of the pulmonary veins empty into the right atrium or one of its tributaries. Emphasis in this study is laid on the value of tomography in detecting the presence of anomalous pulmonary venous connections, in visualizing the detailed morphology, and in demonstrating associated bronchopulmovascular malformations. The tomographic method, including a new technique for viewing the retrocardiac part of the pulmonary veins, was applied extensively to 9 patients. There were 4 different types of the partial, and 1 of the total form of the anomaly. The diverse roentgenographic signs of the anomaly in conventional and tomographic roentgenograms, as described in the literature, are grouped according to changes in

the lung fields and hilar regions, the upper mediastinum, the heart and great vessels, and other thoracic structures. Additional roentgenographic appearances, not hitherto reported, are defined and illustrated.—W. J. STEININGER

Roentgenologic Diagnosis of Rupture of the Diaphragm. O. BARTLEY* and I. WICKBOM. *Acta Radiol (Stockh)*, January, 1960, 53: 33–41. (* Sahlgrenska Sjukhuset, Gothenburg, Sweden)

Observations and data are based on a study of 9 cases of diaphragmatic rupture, 8 of which were on the left side of the diaphragm. The cause of rupture is reported as usually being the result of nonpenetrating trauma to the abdomen. The signs are due to herniation of abdominal organs into the thorax. The condition can easily be overlooked. It is reported that 95 per cent of the cases of rupture occur on the left side and, as a result, the signs of pressure on the stomach, left colon, and spleen make themselves manifest. Intrathoracic pressure increase produces symptoms resembling shock, with dyspnea and circulatory collapse. Digestive tract symptoms of vomiting and retrosternal pain may be present. Total intestinal obstruction may occur. Diagnosis may be suspected by a combination of the above signs and symptoms, but is really established by the chest roentgenogram revealing parts of the stomach or colon in the thorax. Failure to establish the diagnosis is due to a distended stomach or colon being mistaken for a high diaphragm or, less commonly, by pleural fluid concealing the abdominal organs. Barium meal or enema can be used as supplementary examinations even if the patient is in rather poor condition. Pneumoperitoneum is mentioned as a diagnostic aid. Right-sided ruptures are more difficult to diagnose. The signs and symptoms in a right rupture are apt to be less alarming.—E. SHABART

Recognition of Infrapulmonary Pleural Effusion. J. A. PETERSEN*. *Radiology*, January, 1960, 74: 34–41. (* Massachusetts General Hosp., Boston)

Roentgenographic recognition of infrapulmonary pleural effusion on the left side usually is not difficult because of the unusual separation of the lung and the gas bubble of the stomach. On the right side, the most valuable criterion for recognition is the identification on the lateral view of the "straight-line" contour of the anterior portion of the pseudo-diaphragmatic shadow. This image is produced by the fluid adjacent to the posterior surface of the middle lobe. The characteristic curvature of the diaphragm-like shadow seen on the postero-anterior projection is an excellent sign of infrapulmonary effusion, but was present in less than half of the 25 cases in this series. Demonstration by fluoroscopy or on the lateral film of fluid spilling out into the lateral or posterior gutter is also a valuable finding in detection of infrapulmonary effusion. Lateral decubitus and supine studies often show the true level of the diaphragm and mobility of the fluid. Pneumoperitoneum with upright films sharply delineates the level of the diaphragm and indicates the exact position of the fluid, as well as its formation in a curved layer between lung and diaphragm. Suspicion of every elevated diaphragm will be rewarding in leading to the diagnosis of infrapulmonary effusion.—W. J. STEININGER

Chest Roentgenographic Changes in Systemic Lupus Erythematosus. J. G. BULGRIN*, E. L. DUBOIS, and G. JACOBSON. *Radiology*, January, 1960, 74: 42–49. (* U. of S. California School of Med., Los Angeles)

The protean manifestations of systemic lupus erythematosus, variations in clinical material from series to series, differences in roentgen coverage, and perhaps other factors, have resulted in such extreme variability in the reported incidence of roentgenographic changes in the chest that one must beware of drawing conclusions upon the basis of any single study of this disease. Although it is unusual, systemic lupus erythematosus may exist for many years and even terminate fatally without any roentgenographically demonstrable thoracic abnormality. Pleural and cardiac changes are almost equally frequent, and are often combined. Effusions are generally small, with residual thickening the rule. In ⅓ to ½ of the cases, involvement is bilateral. Cardiac enlargement is usually minimal to moderate, and rarely is the etiology apparent. When the disease is manifest in several intrathoracic structures rather than a single one, rapid serial film changes become far more frequent. This may be a reflection of dissemination. Involvement of the lungs is less frequent than that of the pleura or heart, and is not specific for the disease. Interesting rare types of infiltration occur. Three cases are reported. —W. J. STEININGER

Post-Pneumonic Pulmonary Pneumatoceles. R. A. FLAHERTY*, J. M. KEEGAN, and H. N. STURTEVANT. *Radiology*, January, 1960, 74: 50–53. (*Lutheran Hosp., Fort Wayne, Ind.)

Twenty cases are reported in which pulmonary pneumatoceles developed as a complication of pneumonia: 8 in children, 12 in adults. Pulmonary pneumatoceles probably begin as small abscesses, but continue to enlarge and develop as localized areas of obstructive emphysema. Pneumatoceles do not produce symptoms unless they enlarge sufficiently to embarrass respiration. They occa-

sionally contain fluid, but most often are completely air-filled. Pneumatoceles occur during and following pneumonia, usually staphylococcal in origin, and they almost invariably regress spontaneously.—W. J. STEININGER

Roentgenograms of the Chest in Six Cases of Scleroderma (in Japanese). T. KOBAYASHI*, Y. KOBAYASHI, K. NISHIHARA, and Y. HAMAZAKI. *Clin Radiol*, February, 1960, 5: 135–140. (* Kanazawa U. School of Med., Japan)

The roentgenographic changes of the chest are described in 6 patients with scleroderma (5 females and 1 male, aged between 19 and 40 years). The changes were more pronounced the longer the duration of the illness. The main pulmonary changes were best described as fibrocystic patterns, and were usually seen in the lower lung fields. Cardiac enlargement was seen in 3 cases, and conspicuous cardiac enlargement with a cardiothoracic ratio of about 4 to 5 with pleural effusion was seen in 1 of these 3. Complications will alter the pre-existing roentgenographic changes, but the writers emphasize the importance of being acquainted with the basic roentgenographic findings in collagen diseases because these diseases are generalized and afflict many organs. —I. TATENO

Radiologic Aspects of Stab Wounds of the Chest. L. C. DOUBLEDAY*. *Radiology*, January, 1960, 74: 26–33. (* 1917 Ashland, Houston, Texas)

The significance of initial roentgenograms following stab wounds of the chest is discussed with reference to 3 anatomic areas: supraclavicular and mediastinal, pericardial, and lower thoracic. Unsuspected hemomediastinum complicating a supraclavicular stab wound is manifested by a characteristic widening of the superior mediastinum. Usually, as pressure in the mediastinum mounts, the mediastinal pleura ruptures and associated hemothorax develops. In suspected hemopericardium, initial anteroposterior supine projections of the chest are difficult to interpret accurately, but many cases of proved hemopericardium show straightening of the left heart border. Serial films are necessary to detect reaccumulation of fluid and delayed exudative pericardial effusion. In follow-up films, the cardiac silhouette may appear within normal limits even in the presence of appreciable amounts of fluid in the pericardial sac, necessitating angiocardiography. Although aspiration is the treatment of choice initially, opening in the parietal pericardium should be made as a secondary procedure if fluid persists, permitting drainage into the pleural space and avoiding delayed exudative pericarditis and adhesive pericarditis. In penetrating wounds of the left lower thorax, occult injury to the spleen is diagnosed readily by change in contour of the splenic shadow in follow-up films of the chest and abdomen.—W. J. STEININGER

Long-term Follow-up of 22 Patients Irradiated for Thymic Enlargement in Infancy. C. G. H. NEWMAN*. *Brit Med J*, January 2, 1960, No. 5165: 34–36. (* Hosp. for Sick Children, London, England)

Thirty-two patients who received irradiation for suspected thymic enlargement in infancy between 1932 and 1950 are reviewed: 31 have been traced. Three have died, 1 possibly from the acute effects of the radiation. One patient developed a nodular goiter 22 years after the irradiation, another had slight diffuse enlargement of the thyroid at the age of 17, and another had a neurilemmoma removed from the neck at the age of 14. There were no cases of leukemia (after author's summary).—E. A. RILEY

CHEMOTHERAPY

Susceptibility of *Hemophilus Influenzae* to 21 Antibiotics *in Vitro*. H. A. HIRSCH* and M. FINLAND, with C. WILCOX. *Amer J Med Sci*, January, 1960, 239: 33–40. (*Boston City Hosp., Mass.)

Strains of *Hemophilus influenzae* isolated early in 1959, mostly from the respiratory tracts of hospitalized patients, were tested for *in vitro* susceptibility to 21 antimicrobials—including several that had been used previously for similar tests on strains isolated in 1954 and 1949—and, also, several groups of related drugs. All of the antimicrobials were roughly similar in their activity against these organisms except Bryamycin® (a sulfur-containing polypeptide produced by *Streptomyces hawaiiensis*) and some of the erythromycin-like drugs (carbomycin, oleandomycin, spiramycin, and leukomycin) which were much less active, and penicillin, which was more active than all of the others against more than ½ of the strains. The results obtained with the strains isolated in 1959 were similar to those obtained with some of the same antimicrobials in the 2 earlier studies. It is concluded that there has been no significant change in the drug susceptibility of strains of *H. influenzae* isolated in Boston City Hospital during the past 10 years. Most of the newly introduced antimicrobials are about as active against *H. influenzae* as those that were available previously, but some of the erythromycin-like drugs are much less active. Colimycin appears to be slightly less active than polymyxin B, the 4 tetracycline analogues all have very

similar activity, and there are only slight differences in the activity of streptomycin, neomycin, kanamycin, and paromomycin.—W. J. STEININGER

Resistance of *M. Tuberculosis* to Antimicrobials, and Therapeutic Results in the Treatment of Pulmonary Tuberculosis (in French). C. MATTEI*, P. LAVAL, J. DUNAN, and Mme CHARREL. *Rev Tuberc (Par)*, October–November, 1959, 23: 933–948. (*Marseille, France)

The resistance of *M. tuberculosis* has been determined in the laboratory according to the following criteria: diminution of susceptibility (10–20 colonies per cu. cm. in tubes containing 5γ of streptomycin per ml., 1.0γ of isoniazid per ml., or 0.5γ of PAS per ml.); and true resistance (20 colonies per cu. cm. in tubes containing 10γ of streptomycin per ml., 5γ of isoniazid per ml., or 2γ of PAS per ml.). The study comprised 48 patients with total susceptibility to these drugs, 48 patients who showed initial resistance (weak resistance in 18 and true resistance in 30), and 52 patients in whom resistance emerged after repeated and often poorly conducted antimicrobial treatments (weak resistance in 10 and true resistance in 42). The following results were obtained: group I, 91.6 per cent excellent results in recent infiltrations of limited extent; group II, 60.4 per cent very marked improvement under continued chemotherapy; group III, only 8.6 per cent improvement and 27 per cent complete failures. The latter were caused by extensive irreversible lesions and weakened general condition (after authors' summary).—V. LEITES

Chemotherapy in Chronic Bronchitis. R. S. FRANCIS* and C. C. SPICER. *Brit Med J*, January 30, 1960, No. 5169: 297–301. (*Report of the British Tuberculosis Association, London, England)

A controlled trial has been carried out to estimate the usefulness of penicillin and tetracycline as long-term prophylactics in chronic bronchitis. Information was specially collected to determine the monetary losses from the disease in relation to the cost of treatment. It was found that 312 mg. of penicillin V or 250 mg. of tetracycline twice daily approximately halved the days lost from work as compared with a control group receiving inert treatment. Tetracycline did not differ significantly in its effect from penicillin, but the cost of treatment was much greater. Neither drug significantly reduced the number of attacks of bronchitis. The validity of the results is discussed, and it is concluded that the method of study used was reliable (Authors' summary).—E. A. RILEY

Chemotherapy and Intrafocal Tubercle Bacilli (in Japanese). Y. KANO* and S. HAMANO. *Jap J Chest Dis*, February, 1960, 19: 102–110. (*Muramatsu Seiranso Sanatorium, Ibaraki, Japan)

The culturability of tubercle bacilli in the tuberculous lesion differs considerably according to the type of focus (such as cavity, inspissated cavity, or encapsulated focus). In the case of closed pulmonary lesions, the incidence of cultures positive for tubercle bacilli becomes less the longer the duration of chemotherapy. However, when the necrotic tissue is altered in structure, the tubercle bacilli may in more than a few cases persist even when the sputum is free of tubercle bacilli. The cavities which cannot retract to a smaller size because of pleural adhesions, et cetera, are likely to help bacilli acquire resistance to the drugs, and the scope of chemotherapy is ambiguous in these cases. However, the cavities which can retract and turn into a closed lesion before bacterial resistance develops may be amenable to successful chemotherapy. When one considers the biology of tubercle bacilli in the tuberculous foci, the pathologic and anatomic conditions, as well as the effect of chemotherapy, must be taken into consideration.—I. TATENO

A Decade of Chemotherapy of Pulmonary Tuberculosis at the University Hospital of Freiburg (in German). W. SCHIESSLE*, G. BUCHEGGER, H. KILCHLING, and M. HINKEL. *Beitr Klin Tuberk*, June, 1959, 120: 255–273. (*Robert-Koch-Klinik, Freiburg, Germany)

A historic review is presented of the treatment of tuberculosis dating back for some 100 years, with detailed reports on the recent 10 years of chemotherapy at the University Hospital in Freiburg, Germany. More than 1,200 patients were treated with the various antimicrobials starting with thiosemicarbazone and PAS, and continuing with streptomycin, isoniazid, cycloserine, and viomycin. Some of the drugs were administered alone at the beginning of the chemotherapeutic era; later, in a combination of 2 or 3 drugs. Isoniazid–streptomycin has been the best combination when 2 drugs were employed; the therapeutic effect has been enhanced by adding PAS (in infusion) to the combination. However, there still remain tuberculous patients who cannot be cured.—Z. VIRÁGH

Chemotherapeutic Pitfalls in the Treatment of Tuberculosis. G. POOLE* and P. STRADLING. *Brit Med J*, January 16, 1960, No. 5167: 161–166. (*Hammersmith Hosp., London, England)

An analysis of 32 fully documented case histories yielded 59 examples of drug resistance in *M.*

tuberculosis. In 33 (70 per cent) of 47 initially resistant examples, PAS failed to prevent resistance to the other drugs given. By far the most likely explanation for this is that PAS was not consumed in adequate regular amounts. Evidence is also presented that PAS plus isoniazid given together in single cachet form may also not be effective, perhaps for the same reason. No evidence was found that isoniazid is similarly rejected by patients. In 14 examples (30 per cent), faulty prescription of drugs was responsible for the resistance. This included the use of 1 drug alone, prescribing 1 daily and the other intermittently, the use of such unproved substances as B-PAS, the combined form of isoniazid–PAS (Dipasic®) or a thiosemicarbazone and, also, oxytetracycline given with streptomycin. The importance is stressed of: (*a*) using sensitive drug-resistance tests and accepting a low threshold of resistance, (*b*) scrutinizing a patient's previous therapeutic history and obtaining susceptibility reactions when possible before prescribing further antituberculosis drugs in an effort to avoid resistances, (*c*) avoiding the prescription of PAS when its administration cannot be fully supervised, (*d*) never using drugs that have not shown themselves satisfactory in rigidly controlled clinical and bacteriologic field trials (B-PAS; Dipasic; thiosemicarbazones and their combinations such as "Tebafen" and hinconstarch). It is concluded that, until a more satisfactory oral agent can be found to accompany isoniazid, long-term daily streptomycin given with daily isoniazid is not only the most effective combination therapeutically, but the only way of ensuring adequate treatment in spite of the patient's shortcomings. The ease with which major therapeutic errors with tragic consequences can be made is fully stressed. This point is illustrated by the fact that 15 of the 32 patients in whom drug resistance occurred have since died of their disease: this is in stark contrast to the now usual results of correct long-term chemotherapy (after authors' summary).
—E. A. Riley

Roentgenographic and Clinical Results of Ambulatory Chemotherapy of Tuberculosis in Dakar (in French). M. Rouan* and E. Tête. *Rev Tuberc (Par)*, October–November, 1959, 23: 1051–1062.

Of 1,926 patients with pulmonary tuberculosis who were admitted to the Center of Phthisiology in Dakar for ambulatory treatment and who had never been treated in a hospital, 915 were lost from supervision, frequently after the first visit; the reasons were excessive distance of the center from their home, physical exhaustion, resumption of ancestral medications, or recourse to witchcraft. Of the 1,011 patients who remained under care, 324 (32 per cent) were not benefited by treatment: they remained incurable, became progressively worse, or died; 68 per cent were improved by ambulatory treatment, but only 20 per cent had solid results permitting them to resume full-time work. On the other hand, the percentage of good durable results after treatment in the hospital was 73. Therefore, ambulatory chemotherapy in the African milieu is only a last resource. However, in spite of very incomplete results, ⅔ of the patients treated showed sputum conversion after 2–3 months of treatment. In view of the poor socioeconomic conditions, with extreme overcrowding and promiscuity in Dakar and other large urban centers in Africa, this is considered the essential merit of ambulatory treatment. Systematic chemoprophylaxis of contacts of open cases is advised in preference to BCG, which is considered insufficient under the existing conditions.—V. Leites

Fungistatic Action of Diamidinodiphenilamine Dihydrochloride in Deep Mycoses (in Spanish). J. E. MacKinnon*, R. C. Artagaveytia-Allende, and N. Garcia-Zorrón. *Rev Lat Amer Microbiol*, June, 1958, 1: 153–158. (*Instituto de Higiene, Montevideo, Uruguay)

The *in vitro* effect of diamidinodiphenilamine dihydrochloride on various strains of pathogenic fungi can be favorably compared with that of stilbamidine, hydroxystilbamidine, and aminostilbamidine. This aromatic diamidine in a concentration of 10γ per ml. (in some cases, 5γ per ml.) completely inhibited the growth of several strains of *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, and some other fungi. The utilization of the drug in the treatment of fungous diseases is suggested.—Z. Virágh

Corticosteroids in Childhood Tuberculosis (in Spanish). R. Infante* and O. Montes. *Rev Chile Enferm Torax*, No. 2, 1958, 23: 77–97. (*Hosp. Roberto del Rio, Santiago, Chile)

Corticosteroids together with antituberculosis drugs were employed in 39 children aged 5 months to 14 years. Processes subjected to treatment included 13 cases of extrapulmonary—mostly cervical—tuberculous adenitis, 4 primary pulmonary infections with atelectasis, 3 pleural effusions, and 17 cases of meningitis. Of the 17 children with meningitis, 10 had generalized tuberculosis as well. The results were good in all but 1 of the patients with lymphadenitis and 1 among the pulmonary cases. The response was satisfactory in the 3 children with pleural effusions. In the meningitis group, 11 patients improved without sequelae,

1 child developed hydrocephalus, and 5 died. In addition, 2 children—believed to have rheumatoid arthritis—were treated with steroids and salicylates without antituberculosis drugs; the ensuing generalized tuberculosis proved this diagnosis to be erroneous.—Z. VIRÁGH

Local Treatment of Joint Tuberculosis with Corticosteroids (in German). H. WEHRLIN* and S. SEYFFERT. *Schweiz Z Tuberk*, No. 2, 1959, 16: 106–112. (*Davos-Clavadel, Switzerland)

Fifteen patients with joint tuberculosis (knee, shoulder, elbow, and finger) were treated with intra-articular streptomycin and corticosteroid injections parallel with streptomycin, isoniazid, and PAS administered orally in various combinations. The result was good and this method of treatment is recommended. One case history is presented in illustration.—Z. VIRÁGH

Influence of Protracted Isoniazid Treatment on the Fertility of White Mice (in German). P. STEINBRÜCK*. *Z Tuberk*, September, 1959, 113: 281–284. (*Tuberkulose-Institut, Berlin-Buch, Germany)

Therapeutic doses of isoniazid were given to many generations of white mice. From the second generation on, the fertility was reduced significantly. This seemed to be due to disturbance in spermiogenesis, with a lesser effect on female animals. These effects appeared to be reversible. —J. HAAPANEN

Technical Study of Bioassay of Active Isoniazid in Body Fluids by Vertical Diffusion (in French). A. TACQUET* and F. TISON. *Ann Inst Pasteur (Lille)*, January, 1960, 98: 18–27. (*Inst. Pasteur, Lille, France)

Cerebral Complication Following Isoniazid Administration. W. KRAMER*. *Acta Tuberc Scand*, No. 1, 1960, 38: 51–57. (*State U., Leyden, Holland)

Daily administration of 300 mg. of isoniazid and 12 gm. of PAS for 11 weeks to a 16-year-old girl with pulmonary tuberculosis and erythema nodosum was followed by acute signs of cerebral disturbance. After an initial period of severe headache, vomiting, and blurred vision, a status epilepticus developed, followed by a postparoxysmal right hemiparesis. The patient remained unconscious until the fourth day. Soon after cessation of isoniazid and PAS administration, the cerebral symptoms cleared completely, the only traces being electro-encephalographic abnormalities which were still present 6 weeks later. This syndrome was thought to be due to isoniazid intoxication.—W. J. STEININGER

Further Observations on the Isoniazid-Resistance, Catalase Activity, and Virulence of *M. Tuberculosis*. E. VINCZE*, O. SCHWEIGER, and E. VANDRA. *Acta Tuberc Scand*, No. 1, 1960, 38: 26–39. (*National Institute for Tuberculosis "Korányi," Budapest, Hungary)

Isoniazid-resistant and isoniazid-susceptible strains of *M. tuberculosis* were inoculated into guinea pigs and were recultured at various intervals from the organs of the animals. The recultured strains were examined for isoniazid resistance, catalase activity, and virulence. The results were as follows: (*1*) The reculturability of isoniazid-resistant strains showed progressive decrease after infection. Eight weeks after infection, recultures could be obtained in only a few instances. Catalase-positive, isoniazid-resistant strains were recultured more frequently from the organs of the animals than were catalase-negative, isoniazid-resistant strains. The reculturability of mixed populations was identical with that of isoniazid-susceptible strains. (*2*) When catalase-negative, highly isoniazid-resistant strains were used for infection, catalase-negative highly resistant strains predominated among the strains obtained by reculture. However, a certain number of catalase-positive slightly resistant strains also were found. When catalase-positive strains were used for infection, the majority of strains recultured showed various levels of isoniazid resistance. With the progress of the infection, the catalase-positive strains tended to predominate. (*3*) A correlation was found between reculturability from the animals, isoniazid resistance, and virulence of the strains used. Catalase-negative strains possess considerably lowered pathogenicity, as evidenced by spontaneous healing of the lesions after initial progression. A similar tendency of the lesions to regress was not observed with isoniazid-resistant, catalase-positive strains (Authors' summary).—W. J. STEININGER

Experimental and Clinical Study of Kanamycin in Tuberculous Infection (in French). G. BROUET*, J. MARCHE, J. CHEVALIER, F. LIOT, G. LE MEUR, and MME BERGOGNE. *Rev Tuberc (Par)*, October–November, 1959, 23: 949–988. (*Paris, France)

The effects of kanamycin were studied in 70 patients with pulmonary tuberculosis. The disease was of recent onset in 32 and of long duration in 38. Most cases were moderately or far advanced and had cavitation. The sputum was positive in 64. No patient in this series received less than a total dose of 45 gm. of kanamycin (in a daily dose of 1.0 gm.). Because of toxic manifestations, only 26 of the 70 patients could complete the planned course of 90 days and longer. In 24 patients,

kanamycin was given alone; in 46, in combination with other drugs (mostly isoniazid). In 44 patients the urine showed granular casts, most often after 1 month of treatment. Ototoxicity was frequent. Of 67 audiograms, 10 were normal, 2 doubtful, and 55 abnormal. Among the abnormal audiograms, 24 showed rather severe disturbance, 7 severe disturbance, and 24 less important changes. The degree and incidence of auditory impairment was higher in patients previously treated with streptomycin. Ototoxicity was seen even after moderate doses (60 gm.), and became more severe with higher doses. The alterations in the audiograms developed abruptly from one examination to the other and increased rapidly if treatment was not stopped promptly.

Audiograms should be performed every 10 days in all patients receiving kanamycin. Of the 24 patients treated with kanamycin alone, 19 had recent forms of tuberculosis. Very severe cases were not included in this group. The roentgenographic results were very good in 1 case, good in 9, moderate in 6, mediocre in 3, and nil in 5. Twenty-two of the 24 patients had infectious sputum prior to kanamycin treatment; 9 of these had negative cultures at the end of treatment (70–90 days). The total results were very satisfactory in 25 per cent of the patients. This figure would probably have been higher if ototoxicity had not necessitated stopping treatment prematurely.—V. LEITES

Para-Aminosalicylic Acid (PAS) Concentrations in the Serum During Treatment with Various PAS Preparations. K. M. CITRON* and S. W. A. KUPER. *Tubercle*, December, 1959, 40: 443–452. (*Brompton Hosp., London, England)

Serum PAS concentrations during treatment with sodium PAS in solution and cachets have been compared with those obtained during treatment with other preparations of PAS. Preparations of sodium PAS in the form of granules and effervescent tablets and calcium PAS cachets gave serum levels similar to those after sodium PAS cachets. An enteric-coated tablet of sodium PAS gave low levels. Phenyl PAS gave low but steady levels. Phenyl PAS does not produce peak serum PAS levels as great as do corresponding doses of sodium PAS. The safety of phenyl PAS must therefore depend upon information from clinical trials. Preliminary information, though encouraging, cannot be considered conclusive in view of the small numbers of patients so far investigated. In view of the low serum PAS levels produced by calcium benzoyl PAS, and the conflicting evidence about its efficacy from *in vitro* studies and trial in animals and man, it is believed that calcium benzoyl PAS cannot at present be regarded as a satisfactory alternative to sodium or calcium PAS.—M. J. SMALL

Drug Resistance in Out-patients Treated with Sodium PAS and Isoniazid Cachets. J. McD. SIMPSON*. *Tubercle*, December, 1959, 40: 453–456. (*Royal Victoria Dispensary, Edinburgh, Scotland)

The majority of outpatients receiving long-term chemotherapy for tuberculosis are treated with combinations of PAS and isoniazid. The main disadvantages of this method are the relatively high incidence of gastrointestinal intolerance to PAS, and the difficulties experienced in ensuring regular ingestion of drugs by patients out of hospital. The introduction of side-room tests for urinary PAS revealed a greater incidence of irregular medication than had been previously appreciated. An attempt was made to obviate the danger by dispensing the correct proportions of both drugs in the same powder or cachet. That this maneuver may not be sufficient if chemotherapy is taken in a very irregular and haphazard manner is suggested by the occurrence of isoniazid resistance in 12 patients, for whom both drugs had been prescribed together in the same cachets. Ten of the 12 cases were collected at a single large chest clinic over a 4-year-period during which approximately 2,500 outpatients were treated with PAS-isoniazid cachets. In only 1 patient was bacteriologic relapse with the emergence of isoniazid-resistant bacilli not accompanied by roentgenographic deterioration. If a patient fails to take his full number of cachets, even though the dose of isoniazid is reduced proportionately to that of PAS, the amount of the latter drug may in some cases prove insufficient to prevent the emergence of isoniazid resistance.

Owing to the continued administration of both PAS and isoniazid after loss of isoniazid susceptibility, 4 of the 12 patients developed resistance to PAS in addition. Consequently, if relapse, for which there is no other satisfactory explanation, occurs during treatment with PAS and isoniazid in adequate dosages, the drugs should be discontinued temporarily until sensitivity results are obtained. Otherwise, loss of susceptibility to PAS may also develop, as occurred in 4 of the 12 individuals in this series.—M. J. SMALL

Drug Treatment of Experimental Silicosis. D. M. JAMES, T. G. MORRIS, and J. MARKS*. *Brit J Industr Med*, January, 1960, 17: 36–40. (*Central Tuberculosis Lab., Cardiff, Wales)

It has already been shown that tissue cultures of phagocytic cells may be protected against the toxic effect of silica dust by a number of organic bases and aluminum complexes. These compounds

have now been used to treat mice after the administration of silica dust intravenously. Protective activity was measured by the capacity to inhibit or retard the development of silicotic nodules in the livers of experimental animals. Drugs active in this respect were an aluminum-dextran complex, phenazine B749, methylene blue, and compound 46-107. Compound 46-107 is related chemically to compound 48-80, the subject of a previous study of protective action, but lacks the latter drug's disruptive effect on mast cells. (Authors' summary).—H. J. SIMON

Studies on Streptomycin Levels of Blood and Abscess. E. HEVÉR* and T. RISKÓ. *Acta Tuberc Scand*, No. 1, 1960, 38: 40–50. (*"Fodor Jozsef" State Institute for Tuberculosis, Budapest, Hungary)

The standard Oxford auxonographic method was used to determine streptomycin concentrations of blood and of pus in cold abscesses. Intramuscular administration of streptomycin leads to an early rise in blood levels with a rapid fall subsequently. The degree of rise in abscess levels is much smaller, therapeutically effective levels often not being reached. The levels reached in abscesses, however, persist for relatively long periods. Intrafocal (local) injection of streptomycin into the cold abscesses, in addition to producing high concentrations locally, leads to persistently high concentrations in the blood, exceeding significantly the therapeutically effective level. Among the body fluids, the blood showed the greatest reduction in streptomycin concentration as a result of storage.—W. J. STEININGER

2-Ethylisothionicotinamide ("1314") in Pulmonary Tuberculosis: A Controlled Trial of Drug Tolerance. A. R. SOMNER*. *Tubercle*, December, 1959, 40: 457–461. (*U. of Edinburgh, Scotland)

A double-blind controlled trial was carried out in patients with pulmonary tuberculosis to determine the extent of tolerance to enteric-coated 2-ethylisothionicotinamide (TH 1314; thioamide) when given in a dose of 0.5, 0.75, and 1.0 gm. daily. Forty patients took part in a trial lasting 4 weeks, the unit of study being the patient-week. The results showed that nausea was experienced in about ¼ of the patient-weeks with a daily dose of 0.5 gm., in ½ the patient-weeks with 0.75 gm. and 1 gm. daily, and in 1/12 of the patient-weeks on control tablets. Apart from the effect on the alimentary tract, no other important symptoms were noted. The development of significant intolerance to this drug when used in the treatment of patients with tuberculosis is unfortunate as this may limit its usefulness, particularly in the home treatment of tuberculosis. The optimal dose of thioamide in tuberculosis is as yet unknown. —M. J. SMALL

A New Treatment of Upper Respiratory Infections with Trypsin-Phenylephrine Nose Drops. A. J. MAFFIA*, M. LEVBARG, L. A. PERILLO, and W. GREENBERG. *Arch Pediat*, January, 1960, 77: 28–32. (*Flower and Fifth Avenue Hospitals, New York, N. Y.)

A new nasal preparation, Trypp®, which contains buffered trypsin, is described. In the laboratory this preparation is mucolytic, has some local anti-inflammatory properties, and does not impair ciliary function. Clinical studies have indicated that there is practically no irritation or sensitivity with these nose drops. In 202 children with upper respiratory infections or exacerbation of chronic sinusitis treated with Trypp nose drops, there was excellent response in 175 (87 per cent), good results in 23 (11 per cent), and poor or no response in 4 (2 per cent).—M. J. SMALL

*The Medical Letter**, April 29, 1960, 2: 33–36. (*136 East 57th St., N. Y., N. Y.)

Treatment of trichomonal vaginitis; methyprylon (Noludar®), a piperidine derivative, as a "nonbarbiturate" hypnotic; halothane (Fluothane®), a new general anesthetic agent; a polyurethane polymer publicized as a "bone glue" for the treatment of various types of fractures (Ostamer®).

The Medical Letter, May 13, 1960, 2: 37–40.

Methaminodiazepoxide (Librium®) for the treatment of emotional disorders; prophylaxis and treatment of "traveler's diarrhea"; the intramuscular iron-dextran compound, Imferon®, withdrawn from sale at the request of the Food and Drug Administration.

PULMONARY PHYSIOLOGY

The Value of Oxygenation Prior to Induced Apnea. M. FUJIMORI* and R. VIRTUE. *Anesthesiology*, January–February, 1960, 21: 46–49. (*U. of Colorado Med. Center, Denver)

The danger is pointed out of exposing a patient to hypoxia while using apneic techniques during anesthesia induction. A routine to protect a patient against prolonged hypoxia is advocated as being desirable. The electro-encephalogram is used to provide objective information concerning cerebral activity as an indication for impending dangers from hypoxia. Experiments are used to determine the comparative effects of ventilation: (*1*) with air, (*2*) with 100 per cent oxygen for a short period of time, and (*3*) with 100 per cent

oxygen for 30 minutes before apnea. The method is described in detail, using 19 mongrel dogs sedated with sodium pentobarbital and made apneic with succinylcholine. The flat electroencephalogram is used as the criterium to show the presence of dangerous hypoxia. Thirty seconds of pulmonary ventilation with 100 per cent oxygen before production of apnea affords the greater protection and safety. Cardiac irregularities are observed in periods of severe hypoxia.
—E. SHABART

Direct Artificial Respiration (D.A.R.): Present-Day Teaching and Group Training Requirements. M. H. BROOK* and J. BROOK. *Canad Med Ass J*, January 30, 1960, 82: 245–248. (*U. of Saskatchewan, Saskatoon, Canada)

A safe and practical method of demonstration and group training in direct artificial respiration (DAR) is described. This project was undertaken in order to facilitate the adoption of DAR by first aid and civil defense authorities. An extensive program of education is required to place this knowledge in the public domain. This, along with the advances to be made in the field of prevention, will go a long way toward saving some of the many lives that otherwise would be lost as a result of accidents (after authors' summary).—E. A. RILEY

Pulmonary Physiologic Studies on the Effect of Bronchodilator Caytine® (JB-251) (in Japanese). T. YOKOYAMA*, G. EE, H. MATSUMURA, S. NOGUCHI, H. SHINOHARA, and H. KODA. *Clin Resp Organs*, February, 1960, 15: 87–95. (*Keio U. School of Med., Tokyo, Japan)

The effect of a sympathomimetic amine, Caytine® or JB-251, on the ventilatory function was studied in 39 patients with bronchial asthma, 19 with acetylcholine-induced bronchial asthma, 11 with chronic pulmonary emphysema, and 3 patients with bronchiectasis, et cetera, suffering from obstructive ventilatory disturbances. Caytine was given by aerosol inhalation, subcutaneous injection, or by the oral route. On administration of Caytine, there was a decrease in the air-trapping index, and an increase in timed vital capacity and maximal breathing capacity, indicating an improvement in the airway obstruction. Caytine was also effective in some cases refractory to epinephrine and isoproterenol.—I. TATENO

John Snow's Early Research on Carbon Dioxide. R. FOREGGER*. *Anesthesiology*, January–February, 1960, 21: 20–25. (*2373 N. 101st St., Milwaukee, Wisconsin)

As early as 1839, John Snow had performed experiments to show the toxic effects of inhaled carbon dioxide. His experiments showed that carbon dioxide inhaled in an atmosphere low in oxygen could produce fatal conditions. He concluded that concentrations of carbon dioxide of 5 or 6 per cent or higher gave rise to serious symptoms. All of these related historic facts are interestingly presented, using photoduplicates of some of the material as discussed in that period as a result of meetings of the Westminster Medical Society.—E. SHABART

Alveolar Gas Exchange in Clinical Pulmonary Embolism. E. D. ROBIN*, C. E. FORKNER, JR., P. A. BROMBERG, J. R. CROTEAU, and D. M. TRAVIS. *New Engl J Med*, February 11, 1960, 262: 283–287. (* U. of Pittsburgh School of Med., Penn.)

Pulmonary embolism may be considered a disorder affecting the gas-exchange functions of the lung. As a result of changes in gas exchange, 3 major abnormalities occur. One is the development of arterial oxygen unsaturation (3 of the mechanisms for oxygen unsaturation are veno-arterial shunting, decreased diffusing capacity of the lung, and relative alveolar hypoventilation). A second abnormality is the development of hyperventilation. Hyperventilation serves the purpose of assisting normal arterialization of pulmonary capillary blood. The exact mechanisms subserving hyperventilation are not known. The third abnormality is the development of significant differences between the carbon dioxide tension of arterial blood and end-tidal air. This is produced by dilution of alveolar air by the newly formed dead space. Careful studies of gas exchange provide useful information in the diagnosis of pulmonary embolism.

The simultaneous measurements of end-tidal and arterial carbon dioxide tensions provide data of great value in deciding whether surgical intervention is desirable and feasible. The presence of an end-tidal arterial carbon dioxide tension difference indicating that 50 per cent or more of the lung is unperfused, in a patient dying from inadequate gas exchange or inadequate filling of the left side of the heart, is a strong indication for embolectomy. Conversely, an end-tidal arterial carbon dioxide tension difference indicating that less than a main-stem branch is involved is probably a contraindication to embolectomy, since an embolus of this size cannot be readily approached by the surgeon. These criteria are quite preliminary and by no means absolute.
—M. J. SMALL

Further Studies on Impaired Alveolar Function in Chronic Pulmonary Emphysema. Fractional Analysis of CO_2 Tensions in Expired Air and Arterial Blood (in Japanese). T. YOKOYAMA*, G. EE, and H. SASAMOTO. *Resp Circ*, February, 1960, 8: 79–88. (* Keio U. School of Med., Tokyo, Japan)

Fractional analysis of the CO_2 tensions of expired air and arterial blood was performed on 84 patients with chronic pulmonary emphysema. (*1*) The arterial CO_2 tensions in these cases ranged between 34.0 and 74.0 mm. Hg. Abnormally high CO_2 tensions in the arterial blood were seen in 72 cases. (*2*) No linear correlation was found between the CO_2 tensions of fractional expired air and arterial blood. (*3*) The CO_2 tensions of the maximal end-expiratory samples, therefore, may not represent the CO_2 tensions of the arterial blood samples (or of the ideal alveolar air) in some patients with chronic pulmonary emphysema. (*4*) $\Delta'CO_2$, the difference between CO_2 tensions of the maximal end-expiratory air and the arterial blood, ranged between -4.8 and -28.2 mm. Hg. $\Delta'CO_2$ did not reveal any correlation either with other measurements of alveolar ventilation or with other indices of gas distribution. (*5*) ΔCO_2, the difference between CO_2 tensions of the end-tidal air and the maximal end-expiratory air, ranged between 2.1 and 41.5 mm. Hg.; ΔCO_2 did not reveal any correlation with other indices of intrapulmonary distribution of inspired gas. (*6*) It was concluded that perfusion in the presence of impaired intrapulmonary distribution of inspired gas played an important role in bringing about the differences in CO_2 tensions between fractional samples of the alveolar air and the arterial blood. —I. TATENO

Studies on a New Method of Analyzing Intrapulmonary Gas Distribution by the Helium Closed-circuit Method (in Japanese). H. KANAGAMI*, K. SHIROISHI, T. KATSURA, and K. AONUMA. *Resp Circ*, February, 1960, 8: 99–106. (* Kosankin Kenkyujo, Sendai, Japan)

The writers present a new method of analyzing intrapulmonary gas distribution by the helium closed-circuit method in which a changeable volume modification of the closed-circuit method is used. The helium concentration in the lung circuit was measured every 20 seconds in the course of measuring functional residual capacity. The intrapulmonary helium curve was obtained by plotting the helium concentration against the time. This was V-shaped in normal subjects and patients with uniform gas distribution, but was L-shaped in patients with impaired gas distribution from such causes as bronchiectasis, cavitary pulmonary tuberculosis, pulmonary emphysema, et cetera. This curve was analyzed and the value at the point of the lowest helium concentration was named "minimal value." The difference of helium concentration between the minimal value and that after one minute was designated as ΔHe.

The ΔHe and the pulmonary mixing index were measured in 30 normal subjects and patients with noncavitary pulmonary tuberculosis, 11 patients with noncavitary pulmonary tuberculosis, and 27 patients with bronchiectasis. The ΔHe was above 0.5 in the normal subjects and patients with noncavitary pulmonary tuberculosis, but it was below 0.5 in the patients with bronchiectasis and cavitary pulmonary tuberculosis. A significant correlation was found between the ΔHe and the pulmonary mixing index. The ΔHe and the shape of the intrapulmonary helium curve were quite useful in the study of the intrapulmonary gas distribution.—I. TATENO

The Pulmonary Abnormalities in Myxedema. W. R. WILSON* and G. N. BEDELL. *J Clin Invest*, January, 1960, 39: 42–55. (* College of Med., State U. of Iowa, Iowa City)

The diffusing capacity of the lungs for carbon monoxide (D_{LCO}) is reduced in patients with myxedema, and increases slowly but significantly after therapy. The best explanation for this is pulmonary capillary involvement: either a thickened alveolar capillary membrane, or a reduction in the pulmonary capillary bed, or both.
—E. DUNNER

Experimental Verification of the Single Breath Tests of Ventilatory and Ventilation-Perfusion Ratio Inequality. J. B. WEST* and P. HUGH-JONES. *Clin Sci*, 1959, 18: 553–560. (* Hammersmith Hosp., London, England)

The single-breath test of uneven ventilation introduced by Fowler is now widely used. The subject takes a deep breath of pure oxygen which dilutes the alveolar nitrogen concentration to an extent depending on the ventilation in a given region of the lung. He then exhales through a rapid nitrogen analyzer and, since regions with different ventilations do not empty synchronously, the change in alveolar nitrogen concentration during the expiration is a measure of ventilatory inequality. Essentially the same information is obtained if a single breath of an inert gas mixture (for example, argon in air) is given, and this modification has some advantages.

The single-breath test was performed on open-chested dogs in which uneven ventilation and perfusion were obtained at will by tightening screw

clamps on the bronchus and branch of the pulmonary artery to the left lower lobe. The production of uneven ventilation and/or perfusion resulted in the expected changes in the calculated percentage inequalities of ventilation, ventilation-perfusion ratio, and perfusion, provided that some bronchial obstruction was present so that the affected lobe did not empty synchronously with the remainder of the lung. When all parts of the lung emptied simultaneously, no inequality could be detected even when perfusion inequality was present.—H. J. SIMON

Comparative Functional Studies in Cases of Exudative Pleurisy Treated with and without Corticoids. I. HUTÁS* and G. NYIREDY. *Acta Tuberc Scand*, No. 1, 1960, 38: 12–17. (* University Med. School, Budapest, Hungary)

Respiratory function studies and roentgenographic estimates of pleural residue were carried out on 40 patients with acute exudative tuberculous pleurisy, 20 of whom received antituberculosis drug therapy only, the other 20 receiving in addition one month of corticosteroid therapy (20–25 mg. of cortisone per day and 20 units of corticotropin per week, or 10–25 mg. of prednisone). The earlier corticosteroid therapy was introduced, the better were the results obtained. Steroid therapy reduced the frequency and extent of parietal and diaphragmatic adhesions and "callus" formation. The maximal breathing capacity was 30 per cent higher in the group given cortisone. The Tiffeneau number (per cent of vital capacity expired within the first second) was only slightly below normal in the cortisone-treated group (80.5), but definitely pathologic (65) in the controls. Corticosteroid therapy is, therefore, indicated in the treatment of exudative tuberculous pleurisy in conjunction with antimicrobials.
—W. J. STEININGER

Studies of Lung Volume and Physical Working Capacity during Pneumothorax Treatment. C-A. ADAMSON*, S-O. ALBÄCK, H. DAHLSTRÖM, L. EHRNER, and S. E. SJÖGREN. *Acta Tuberc Scand*, No. 1, 1960, 38: 1–11. (* Serafimerlasarettet, Stockholm, Sweden)

In 21 males and 22 females under treatment with artificial pneumothorax because of pulmonary tuberculosis, roentgen examination, spirometry, and working tests on a bicycle ergometer were performed before and after a pneumothorax refill. The pneumothorax refill did not appreciably influence the working capacity. In many patients the respiratory rate during work was high, especially in those with a low maximal breathing capacity. The refill resulted in some cases in an increase, and in other cases in a decrease of functional residual capacity. In subjects who had an increase of functional residual capacity, there was only a slight mediastinal shift after the refill. A marked mediastinal shift occurred in patients in whom the functional residual capacity decreased after the refill. The mediastinal shift started when the pneumothorax pressure rose to values near atmospheric pressure during slow expiration. There was a tendency toward an increase of the functional residual capacity and the residual volume chiefly in patients who had a positive pressure in the pneumothorax cavity throughout the breathing cycle after the refill. Since pneumothorax therapy is rather harmless from the functional point of view and does not seem to prejudice the patient's fitness for work, is reversible, leaves no disfiguring scars, and brings about healing of tuberculous lesions, it still has a place in the treatment of tuberculosis.—W. J. STEININGER

Effect of Deep and Quiet Breathing on Pulmonary Compliance in Man. B. G. FERRIS, JR.*, and D. S. POLLARD. *J Clin Invest*, January, 1960, 39: 145–149. (* Harvard School of Public Health, Boston, Mass.)

Convalescent poliomyelitic patients and normal subjects showed a decrease in their pulmonary compliance as measured in the tidal-volume range during quiet breathing after a series of deep breaths. This change occurred in the prone, supine, lateral, and sitting positions. Compliance decreased 26 to 40 per cent. Two or more deep breaths to the limit of inspiration, after the period of quiet breathing, produced an increase in the compliance; this increase could be eliminated by forced expirations in the normal subjects. It was thought that these changes were probably due to the opening and closing of various units within the lung.—E. DUNNER

Studies of Regional Lung Function Using Radioactive Oxygen. N. A. DYSON*, P. HUGH-JONES, G. R. NEWBERY, J. D. SINCLAIR, and J. B. WEST. *Brit Med J*, January 23, 1960, No. 5168: 231–238. (* Hammersmith Hosp., London, England)

The cycloton-produced radioactive isotope oxygen-15, with a half-life of 2 minutes, has been used to study regional lung function. There is no intubation of the patient. Pairs of scintillation counters placed on the chest wall have been used to measure radiation from 2 regions simultaneously. From observation of counting rates during breath-holding following a single inspiration of active gas, the relative ventilation and blood-flow of the regions are assessed. This paper describes

the results obtained in selected patients with abnormal lung function (Authors' summary). —E. A. RILEY

Effects of Position on Expiratory Reserve Volume of the Lungs. A. B. CRAIG, JR.* *J Appl Physiol*, January, 1960, 15: 59–61. (U. of Rochester, School of Med. and Dentistry, New York)

The expiratory reserve volume (ERV) reflects the resting position of the lungs and thorax and is the most variable subdivision of the lung volume. It was confirmed that the ERV decreased when the subject changed from the sitting to the supine position. When the subject's elbows were supported on the arms of the chair, the ERV increased 3.1 per cent, and a further increase was noted when the subject leaned forward in the sitting position. The maximal ERV was recorded in the hands-knee position. To explain these changes, the effect of the weight of the shoulder girdle and of the abdominal contents must be considered. There may also be other effective elastic forces in the abdomen in addition to those elastic forces contributed by the lungs and thorax (Author's summary).—A. L. L. BELL, JR.

Studies on the Collagen and Elastin Content of the Human Lung. J. A. PIERCE* and J. B. HOCOTT. *J Clin Invest*, January, 1960, 39: 8–14. (* U. of Arkansas Med. Center, Little Rock)

Collagen and elastin were measured in the right middle lobes of 48 people by a modification of the Lowry, Gilligan, and Katersky alkaline-extraction method. Although an increase in elastin occurred with advancing age, there was no significant change in lung collagen. The total collagen and elastin content of the right middle lobes was similar in men and women, although the weight of the lobes was lower in women. —E. DUNNER

Lung Volumes of Singers. S. S. HELLER*, W. R. HICKS, and W. S. ROOT. *J Appl Physiol*, January, 1960, 15: 40–42. (*College of Physicians and Surgeons, Columbia U., New York, N. Y.)

Lung volume determinations (tidal volume, inspiratory capacity, inspiratory reserve volume, expiratory reserve volume, vital capacity, maximal breathing capacity, functional residual capacity, residual volume, and total lung capacity) were carried out on 16 professional singers and 21 subjects who had had no professional vocal training. No differences were found between the two groups of subjects, whether recumbent or standing, which could not be explained on the bases of age, size, or errors involved in making the measurements (after authors' summary). —A. L. L. BELL, JR.

MICROBIOLOGY AND IMMUNOLOGY

Comparative Study of *Mycobacterium Marinum* and *Mycobacterium Balnei* (in Spanish). L. F. BOJALIL*. *Rev Lat Amer Microbiol*, September 30, 1959, 2: 169–174. (*Unidad de Patología, Escuela de Medicina, México 7, D. F.)

Mycobacterium marinum was isolated by Aronson in 1926 from necrotic areas of the liver of certain salt-water fish in which it caused tuberculosis. *Mycobacterium balnei* was identified by Nõrden and Linell (1951) in skin ulcerations of patients who bathed in fish ponds 3 weeks prior to the appearance of the lesions. The microorganism was described as a new species. Investigated by a battery of tests, the 2 mycobacteria proved to be identical—a photochromogenic acid-fast microorganism. Orange-pigment production was observed on Löwenstein medium only. Due to priority, the term *Mycobacterium marinum Aronson* (1926) is suggested for the nomenclature, *M. balnei* to be used as a synonym.—Z. VIRÁGH

Clinical Importance of the Catalase Activity of *Mycobacterium Tuberculosis* (in German). I. SERI* and P. CZANIK. *Beitr Klin Tuberk*, June, 1959, 120: 241–247. (* Korányi Tbc. Intézet, Budapest, Hungary)

Investigating the catalase activity of 103 patients—employing Middlebrook's original method —there were 28 catalase-negative, 48 catalase-positive, and 27 "mixed" secretions. Specimens yielding only catalase-negative bacilli in every examination during a 3-month period were considered "catalase negative"; the opposite were called "catalase positive." When the culture of a certain patient was intermittently catalase positive and negative, it belonged to the "mixed" group. The secretions of each patient who received a minimum of 50 gm. of isoniazid during the treatment time of 3–9 months were subjected to this investigation. Catalase-negative bacilli were observed only (at least, mainly) in the secretions of patients with stationary tuberculous processes; however, the catalase negativity of these bacteria does not necessarily mean that the patient is on the road to recovery, although his chances are better.—Z. VIRÁGH

The Relationship of the Degree of Dispersion in Tween Cultures of *Mycobacterium Tuberculosis* to the Variability in the Numbers of Bacteria in Injection Samples. M. W. BENTZON* and B. MACKEPRANG. *Acta Path Microbiol Scand*, Fasc. 1, 1960, 48: 73–79. (* State Serum Institute, Copenhagen, Denmark)

On the basis of theoretic considerations, it is shown that in animal experiments using Tween®

cultures of *M. tuberculosis* of different degrees of dispersion: (*1*) The greater the degree of dispersion, the less the variability in the number of bacteria with which the different animals are injected. (*2*) With doses in which the average number of bacterial units per volume administered is $\geqq 4$, the probability that the individual animals are injected with bacteria is 98 per cent. However, the number of bacteria required to ensure this degree of certainty is proportional to the degree of dispersion in the suspension.—E. DUNNER

Fluorescence Microscopy in the Study of *Mycobacterium Tuberculosis* and Its Use in the Clinic (in German). B. ADAMCZYK*. *Tuberkulosearzt*, December, 1959, 13: 811–819. (* Wiltbergstrasse, Berlin-Buch, Germany)

During effective antimycobacterial treatment, the tubercle bacillus undergoes morphologic changes which can be visualized under the fluorescent microscope. From the presence or absence of such changes, conclusions can be drawn as to the efficacy of the antituberculosis treatment. This method was used in 1,500 cases.—J. HAAPANEN

Comparative Study of Three Media in Culturing *Mycobacterium Tuberculosis* (in Spanish). J. M. GUTIÉRREZ-VÁZQUEZ*. *Rev Lat Amer Microbiol*, June, 1958, 1: 125–136. (* Escuela Nacional de Ciencias Biológicas, México, D. F.)

The growth-promoting faculties were tested of Löwenstein-Jensen-Holm, Tarshis penicillin–blood-agar, and Hirsch charcoal-agar media by seeding on them 17 specimens (14 sputum, 3 gastric washings) known to be rich in tubercle bacilli. From each specimen 8 cultures decreasing in dilutions by 1 to 10 were planted. The greatest number of positive cultures was obtained by using the Löwenstein-Jensen-Holm medium; however, the Tarshis medium gave earlier results. The Hirsch medium proved to be inferior in these respects. Employing pure cultures of the H37Rv strain in the same dilutions instead of natural specimens from patients, the results were similar in all 3 media.—Z. VIRÁGH

A Simplified Test Employing Egg-containing Medium for Kanamycin Resistance in Tubercle Bacilli (in Japanese). M. TSUKAMURA*. *Kekkaku*, February, 1960, 35: 109–112. (* Obuso Sanatorium, Aichi Prefecture, Japan)

The writer has advocated the use of the same medium for both the isolation of tubercle bacilli and testing for their drug resistance. Egg-containing medium has been rejected by some workers because it gave higher values in tests for kanamycin resistance than did media without egg. For clinical purposes, however, the author asserts that egg-containing "1 per cent Ogawa medium," with and without 100γ of kanamycin per ml., is useful as a medium for testing kanamycin susceptibility. One loopful (0.02 to 0.025 ml.) of sputum digested with an equal amount of 5 per cent potassium hydroxide is inoculated onto the medium, and observations are made after 4 to 6 weeks. When no colonies develop on the medium with 100γ of kanamycin per ml., kanamycin may be expected to be clinically effective. None of the strains of tubercle bacilli from patients previously untreated with kanamycin grew in this medium, although a few strains grew at 50γ per ml. When some colonies develop on the medium with kanamycin, less clinical effectiveness may be expected. When the number of colonies on the medium equals that of the control medium, resistance to kanamycin is evident.—I. TATENO

Further Evaluations of Routine Bacteriologic Methods in the Diagnosis of Tuberculosis. J. WASSERMAN* and G. SVARTZ-MALMBERG. *Acta Path Microbiol Scand*, Fasc. 1, 1960, 48: 31–34. (* Central Bact. Lab. of Stockholm City, Sweden)

Substitution of alkali for acid in the treatment of samples increased the efficiency of the culture method. With bacteria-rich material, this method was as sensitive as guinea pig inoculation. Increase of inoculum volume improved the culture results of gastric washings. The results of the culture of sputum and urine samples remained essentially unchanged. This was probably due to different contamination rates in the different kinds of specimens. In urine samples, alkali treatment reduced the incidence of contaminations when compared to acid treatment. The Sula method for isolation of tubercle bacilli in pleural exudates proved very valuable and comparable to guinea pig inoculation.—E. DUNNER

Microbiologic Procedures of Value in Tuberculosis. G. MIDDLEBROOK*, M. L. COHN, W. E. DYE, W. F. RUSSELL, JR., and D. LEVY. *Acta Tuberc Scand*, No. 1, 1960, 38: 66–81. (* National Jewish Hosp., Denver, Colo.)

The two basic principles for prescribing a predictably successful chemotherapeutic regimen for tuberculous patients are outlined: (*1*) appropriate drugs to which the patient's bacilli are found to be susceptible by drug-sensitivity testing *in vitro*, and (*2*) adequate dosages of these drugs, sufficient to produce sterilizing concentrations at the sites of bacterial multiplication, as determined by microbiologic assays for serum drug levels. By the use of these criteria in pre-

scribing the therapeutic regimens, excellent short-term results (bacteriologic conversion of sputum) and long-term results (prevention of relapses) were achieved in 153 consecutively admitted patients excreting isoniazid- and streptomycin-susceptible bacilli, whose drug dosages were determined by microbiologic assay so as to produce the minimal drug level consistent with bactericidal effect. The technical procedures of these tests are described.—W. J. STEININGER

Comparative Evaluation of Culture and Animal Inoculation in Tuberculosis (in Hungarian). L. LUGOSI*. *Tuberkulozis*, August, 1959, 8: 176–178. (* Országos Korányi Tbc. Intézet, Budapest, Hungary)

Two hundred specimens (sputum, urine, et cetera) were investigated simultaneously by guinea pig inoculation and culture, using 2 liquid and 2 solid media in each case. The results obtained by culture were 1 per cent greater than those rendered by animal inoculation. However, in 2 per cent of the cases, only the animal inoculation was positive, due to the fact that there are strains which lost their pathogenicity: they still grow on culture but are too weak to cause disease.
—Z. VIRÁGH

Studies on Fractions of Methanol Extracts of Tubercle Bacilli. I. Fractions Which Increase Resistance to Infection. C. A. WILLIAMS, JR.*, and R. J. DUBOS. *J Exper Med*, December, 1959, 110: 981–1004. (* The Rockefeller Institute, N. Y., N. Y.)

It is well known that vaccination with killed tubercle bacilli increases the resistance of laboratory animals to experimental tuberculosis. The present report describes in detail the chemical purification, characterization, and vaccinating efficacy of substances obtained from methanol extracts of tubercle bacilli (H37Rv).

Mice of the Rockefeller Swiss-albino strain were injected with the chemically derived vaccines, and subsequently challenged intravenously with a virulent strain of bovine tubercle bacilli (Vallée) to determine the effect of vaccination upon survival time after tuberculous infection. Comparable groups of vaccinated animals were challenged with staphylococci. Some of the vaccine preparations yielded by the fractionation procedure had characteristics of lipopolysaccharides and were capable of inducing resistance to experimental tuberculosis in mice. A mixture in small amounts of 2 of the partially purified fractions afforded greater protection than when either was given alone in larger amounts. Two types of protective activity were observed: (*a*) peak resistance was obtained against both tuberculous and staphylococcal infections between 1 and 3 weeks post vaccination, but decreased thereafter; and (*b*) a second peak resistance against tuberculous infection occurred 6 weeks after vaccination and continued undiminished throughout the 12-week post-vaccination period.

It was postulated that at least 2 independent and possibly supplementary mechanisms are involved in resistance to experimental tuberculosis, 1 exhibiting broad specificity in resistance, and a second producing more specific and longer-lasting immunity. A detailed analysis of this concept is presented in the report.—C. H. PIERCE

A Preliminary Report on an Antimycobacterial Serum Test. A. M. FISCHER* and J. CAUSTON. *Bull Johns Hopkins Hosp*, December, 1959, 105: 360–369. (* Johns Hopkins School of Med., Baltimore, Md.)

To determine whether a combination of antimicrobial agents is synergistic, additive, or antagonistic in its effect upon mycobacteria from tuberculous patients, and to study serum levels, the following test is described: a Tween®-albumin culture of tubercle bacilli isolated from the patient is inoculated into serially diluted serum samples obtained before and at various intervals during antimicrobial therapy. The presence or absence of growth (after incubation for 2 weeks at 37°C.) indicates the effectiveness of the serum levels of the drugs.

The results from a series of tests upon 30 patients with far advanced pulmonary disease were correlated with the apparent response to therapy. Abundant growth of tubercle bacilli was observed in all serum obtained before therapy. After drug administration, inhibition of growth at high dilutions of serum corresponded as a rule with favorable clinical response to treatment, and no inhibition of growth, even at low serum dilutions, corresponded with poor response. Moreover, with any given patient, the effective serum concentrations of drugs at various intervals after administration were reflected in the serum dilution endpoint. Spinal fluid samples obtained from 3 patients with tuberculous meningitis and assayed simultaneously with the respective serum demonstrated that growth-inhibitory levels were comparable in both types of specimens. The results obtained indicate that an antimycobacterial serum test may be of value as a supplement to other laboratory procedures and clinical findings in the therapeutic evaluation and prognosis of the tuberculous patient.—C. H. PIERCE

EXPERIMENTAL PATHOLOGY

Effect of Dietary Proteins and Amino Acids on the Susceptibility of Mice to Bacterial Infections. R. W. SCHAEDLER* and R. J. DUBOS. *J Exper Med*, December, 1959, 110: 921–934. (* The Rockefeller Institute, N. Y., N. Y.)

The previous findings that the quantitative aspect of protein- and amino-acid nutrition is an important factor in the response of mice to bacterial disease have been extended to show the equal importance of the qualitative nature of these dietary constituents. Young mice of the Rockefeller Swiss-albino strain were maintained on experimental diets for various periods before and after infection. The effect of the different dietary regimens was assayed in terms of survival time after inoculation with *M. tuberculosis* var. *bovis*, *M. fortuitum*, *Staphylococcus aureus*, or *Klebsiella pneumoniae* via intravenous, intraperitoneal, or air-borne routes.

Susceptibility to infection was increased when casein, the sole source of amino acids in the diet, was decreased from 20 per cent to 5–8 per cent. This effect upon host resistance was counteracted by the addition of a proper amino-acid mixture to the low casein diet, but not by supplementation with an unbalanced mixture. Wheat gluten or soybean alpha-protein, 15–20 per cent, were ineffective substitutes for the 20 per cent casein diet. Moreover, addition of lysine to the gluten diet did not alter the infection-promoting property even though noninfected animals showed marked improvement in weight gain. Susceptibility to infection was not increased, however, when a mixture of soybean and rice flour providing a protein concentration of 15 per cent and an amino-acid pattern similar to that of casein was employed as the experimental diet. Similar dietary influences upon infection were noted with another strain of albino mice (RFVL) which has a lower natural resistance to infection.—C. H. PIERCE

Experimental Studies in Byssinosis. A. BOUHUYS*, S-E. LINDELL, and G. LUNDIN. *Brit Med J*, January 30, 1960, No. 5169: 324–326. (* University Hosp., Leyden, The Netherlands)

Inhalation of an extract of cotton dust caused a delay in the clearance of nitrogen from the lungs during oxygen breathing. This effect, which presumably is due to bronchial obstruction, was obtained in 2 men who had not previously been exposed to the dust in cotton mills. The effect was not caused by histamine present in the extract. The results of these experiments, as well as some of the symptoms of byssinosis, may be explained by the assumption that the cotton dust contains a histamine liberator. Some evidence was obtained, from animal preparations, that the cotton-dust extract used here contained a substance capable of releasing histamine (after authors' summary).—E. A. RILEY

Malignant Cells in a Peripheral Blood Smear: Report of a Case. G. C. FINKEL* and G. H. TISHKOFF. *New Engl J Med*, January 28, 1960, 262: 187–188. (* Harvard Med. School, Boston, Mass.)

A case is reported of undifferentiated carcinoma of the lung with metastasis to the dorsal vertebra, and with the presence of exfoliated tumor cells in a peripheral blood smear. Diagnostic studies during the patient's hospital course included 2 cytologic sputum examinations, 2 scalene lymph node biopsies, bone-marrow aspiration, and liver biopsy. These were unrevealing. The patient could not tolerate bronchoscopy. Although a search for tumor cells in a peripheral blood smear would probably be unrewarding in most cases, the present case points to the clinical importance of examination of the blood for malignant cells by various concentration techniques in patients in whom malignancy is suspected but other diagnostic methods have been unfruitful.
—M. J. SMALL

Fluorescence Microscopy in Exfoliative Cytology: An Evaluation of Its Application to Cancer Screening. W. UMIKER*, L. PICKLE, and B. WAITE. *Brit J Cancer*, September, 1959, 13: 398–402. (* V. A. Hosp., Ann Arbor, Mich.)

After staining with acridine orange, 1,295 exfoliative cytologic specimens of sputum, bronchial secretions, serous cavity fluids, oral smears, urine, and gastric and esophageal washings were examined by blue-violet fluorescence microscopy. The same smears restained by the Papanicolaou technique provided the controls. The mean examination time per specimen (average of 3 smears per specimen) was 3.8 minutes by the acridine-orange fluorescence technique, and 8 minutes by the Papanicolaou method. Of the 231 specimens found positive by the Papanicolaou method, 191 (83 per cent) were detected by fluorescence microscopy.

The diagnosis of malignancy by the Papanicolaou technique depends mainly on nuclear characteristics; in fluorescence microscopy, the brilliant fluorescence of the cytoplasm is of equal or greater diagnostic value. Not all of the malignant cells exhibited increased fluorescence. This was especially true of some well-differentiated malignant squamous cells. The advantages of fluorescence microscopy in exfoliative cytology are these: (*1*) the staining technique is simple, rapid, and inexpensive; (*2*) scanning for less than 5 minutes

per *set* of smears will detect the majority of specimens containing malignant cells; (*3*) most malignant cells can be recognized at a glance; (*4*) unsatisfactory specimens are recognized readily; and (*5*) the same smears can be restained by standard techniques. Disadvantages include somewhat lesser reliability than the Papanicolaou method (with the possible exception of serous cavity fluid), the necessity for additional equipment and a darkened room, impermanence of smears, possible additional eye strain, and the report of equally good results by the rapid scanning of Papanicolaou-stained smears.—W. J. STEININGER

Changes in the Lung Lipids of Rabbits and Guinea Pigs Exposed to the Inhalation of Silica Dust. G. S. MARKS* and L. W. MARASAS. *Brit J Industr Med*, January, 1960, 17: 31–35. (* South African Institute for Med. Research, Johannesburg)

Rabbits and guinea pigs were exposed to a cloud of silica dust containing approximately 40,000 parts per ml. (0.3–3.0 μ), and were killed at 4-week intervals up to 30 weeks. The guinea pigs showed an increase of lung lipid and phospholipid; the latter showed a parallel with the rising collagen level estimated chemically. In the rabbits there was no increase of either lipid or phospholipid. The likely explanation is that the silica did not reach the lungs in sufficient quantity.
—H. J. SIMON

Studies on the Nature of Silicosis. A Suggested Mechanism of Fibrogenesis. P. F. HOLT and C. W. WENT*. *Brit J Industr Med*, January, 1960, 17: 25–30. (* Reading U., England)

One theory which seeks to explain the production of fibrous tissue in silicosis postulates the interaction of collagen precursors with polysilicic acid. This stage has been questioned because it is doubtful whether polymerized silicic acid is normally formed when quartz dissolves in aqueous media. A mechanism by which silicic acid may be polymerized *in vivo* has been demonstrated by studying the properties of polyamide monolayers, which behave like collagen monolayers when spread on silicic acid substrates. The silicic acid which is adsorbed beneath the monolayer polymerizes when the polyamide films are subjected to pressure. In fibrogenesis, the polysaccharides which are produced in large amounts may serve to prevent the premature agglomeration of the collagen units. Later, when the concentration of the polysaccharide is largely reduced, the polysaccharide may assist in aligning and cementing the units together to form fibers. The physiologic process is probably reversible, but if silicic acid takes the role of the polysaccharide in this second stage, the building up of fibers is likely to be an irreversible process.—H. J. SIMON

Study of Tuberculin Allergy and Infra-allergy to Bacillary Bodies in Their Relationship to Antituberculous Resistance in Guinea Pigs Vaccinated with Large Doses of Oral BCG (in French). L. NÈGRE*, J. BRÉTEY, and MME D. ROY. *Ann Inst Pasteur* (*Par*), January, 1960, 98: 1–6. (* Pasteur Institute, Paris, France)

Adult guinea pigs were vaccinated with 5 consecutive oral doses of 50 mg. of BCG every 4 days. The intradermal reaction to tuberculin was positive in 14 per cent, and to killed BCG in 39 per cent, a total of 45 per cent positive reactions. Post-vaccination allergy appeared only in the second or third months after vaccination; it often could only be demonstrated by a skin reaction to bacillary bodies, showing the weak intensity of allergy. In animals reacting to tuberculin as well as in those reacting to bacillary bodies, the proportion of positive reactions increased until the fifth month, then decreased between the seventh and ninth months. At varying intervals after vaccination, guinea pigs and controls were infected with 0.001 mg. of a virulent bovine strain. In vaccinated animals, the lesions in the organs were extremely rare; in controls, the lungs, livers, and spleens showed numerous confluent lesions. The resistance to challenge infection in guinea pigs thus immunized with the method of A. de Assis was as high as that following parenteral administration of BCG. In guinea pigs vaccinated orally with high doses of BCG, antituberculous resistance was the same in the following 3 categories: guinea pigs reacting or having ceased to react to tuberculin; those reacting or having ceased to react to BCG; and those never having reacted to tuberculin or BCG. These experiments provide additional proof of the complete dissociation of the phenomena of allergy and immunity.
—V. LEITES

Relationship Between Survival Time and Infecting Dose in Red Mice Infected with Coarse and Finely Dispersed Suspensions of Bovine Tubercle Bacilli Dispersed by Means of Ultrasonics. A. JESPERSEN*, B. MACKEPRANG, and M. BENTZON. *Acta Path Microbiol Scand*, Fasc. 1, 1960, 48: 61–72. (* State Serum Institute, Copenhagen, Denmark)

Groups of red mice (20 animals in each group) were infected intravenously with varying numbers of viable bacteria of a 7-day-old Tween® culture of virulent bovine tubercle bacilli. Half of the groups were infected with bacterial suspensions not exposed to ultrasonics (coarse), and the

other half with suspensions exposed to ultrasonics for 10 minutes (finely dispersed). The groups of animals infected with less than about 10^6 viable bacteria from the coarse suspensions lived longer than those infected with the same doses from the finely dispersed suspensions.—E. DUNNER

PUBLIC HEALTH AND EPIDEMIOLOGY

The Risk of Tuberculous Infection in Children (in French). E. ARNOLD*. *Schweiz Z Tuberk*, No. 3–4, 1959, 16: 126–133. (* Sanatorium Genevois, Montana/VS, Switzerland)

In recent years, tuberculous primary infection has occurred mostly in older age; consequently, young children and adolescents remain tuberculin negative. When these young persons become exposed to tuberculous infection in the nursery home or school, the danger of sustaining the disease is greater than was true previously. An epidemic in a nursery home is reported. Of 90 children there were 88 who were tuberculin negative on entrance. A source of infection among the personnel caused positive tuberculin reactions in 36 children, 26 of whom demonstrated primary infections. As a preventive measure, BCG vaccination is advocated.—Z. VIRÁGH

Observations on 130 Young Adults with Primary Tuberculosis (in French). M. GILBERT* and A. DEPRAZ. *Schweiz Z Tuberk*, No. 3–4, 1959, 16: 155–165. (* 21, Rue Toepffer, Geneva, Switzerland)

The average time of observation was 10 years, during which period 76 patients (military personnel) presented 1 or more pulmonary or extrapulmonary complications. These developed most often in the first 2 years. Seven patients died. This report is an argument for BCG vaccination; furthermore, for prolonged antimicrobial treatment of primary tuberculosis.—Z. VIRÁGH

Leukemia and Tuberculosis (in German). G. NEUMANN*. *Beitr Klin Tuberk*, June, 1959, 120: 290–304. (* Stuttgart-S, Schickhardtstr. 35/37, Germany)

(This is the second publication on the subject by this author; the first appeared in this journal in 1958, 118: 348–378.)

Reviewing the German and foreign literature, a definite increase in leukemia is noted—although there are authors who hold the opposite opinion. In the U.S.A. an increase is reported, but only in the adult population, greater in the Negro than in the white; however, the leukemia incidence in children less than 1 year of age decreased. Irradiation as a consequence of diagnostic examination is not considered a factor in the increase of cases. The reason for the greater incidence can be found in the attention being directed toward recognizing the disorder, improved diagnostic methods, and medical care.

World-wide investigations did not reveal a significant increase in leukemia incidence among patients with pulmonary tuberculosis although they are most extensively subjected to diagnostic ionizing radiation. It is possible that a limit exists within which the pathogenic effect of radiation is nil; the likely amount of radiation exposure during diagnostic examination of the thorax lies below this limit. The leukemogenic effect of ionizing radiation is not questioned; however, its importance is overemphasized. The increase of leukemia cases cannot be overlooked; it is noticeable mostly in older people.—Z. VIRÁGH

Skin Allergy to Metabolic Products of Different Mycobacteria (in German). C. SIMON*. *Tuberkulosearzt*, October, 1959: 694–704. (*Kinderklinik, Kiel, Germany)

Intradermal tests with different types of tuberculin were performed in 30 children with latent or manifest tuberculosis and in 1 child with cervical lymphadenitis caused by an unclassified strain. The types used were: Old Tuberculin, purified tuberculin Hoechst, albumose-free avian tuberculin, albumose-free preparations of *M. smegmatis*, *M. lacticola*, *M. phlei*, paratuberculosis, vole bacillus, and BCG vaccine. Nonspecific reactions, i.e., positive reactions to nonmammalian tuberculins, were observed in a number of cases. This confirms the observations made in veterinary medicine that the tuberculin reaction is group-specific, and that cross reactions occur between homologous and heterologous tuberculins. The diagnosis of nontuberculous mycobacterial infections (e.g., *M. ulcerans*, *M. fortuitum*, *M. balnei*) by tests for hypersensitivity still presents difficulties. The positive reactions to OT and to purified tuberculin observed in most mycobacterial infections may be interpreted as group reactions, but they may also be evidence of an undiagnosed healed infection with mammalian tubercle bacilli, or they may indicate a mixed infection with saprophytic strains and noncultivable *M. tuberculosis*. In proved cases of nontuberculous mycobacterial infection, the presence of sensitivity to human tuberculin does not necessarily indicate a tuberculous infection (after author's summary).—J. HAAPANEN

North American Blastomycosis. E. HARRELL* and A. CURTIS. *Amer J Med*, November, 1959, 27: 750–763. (*U. of Michigan Med. Center, Ann Arbor)

There is increasing evidence of occurrence of a subclinical pulmonary form of North American blastomycosis, resembling the benign pulmonary form of coccidioidomycosis and histoplasmosis, which may be more prevalent than is generally thought. It is probable that North American blastomycosis is acquired by inhalation of the organism, and that all patients with the disease so acquired have the potential for systemic spread. The only true cutaneous form of the disease is that rare type which is acquired by primary inoculation of *B. dermatitidis* through the skin. Such an instance is recorded.

The results of intravenous amphotericin B therapy in 8 patients with systemic North American blastomycosis are recorded. The first 4 of these patients were previously reported, and this report constitutes a follow-up. Five of the 8 patients had proved resistant to massive aromatic diamidine therapy; all 8 responded to amphotericin B therapy. Three of the patients have had recurrences following amphotericin B therapy, although they have been clinically free of recurrence for more than 1 year to date. Five of the 8 patients received a total dose of 1 gm. of amphotericin B, 1 received a total dose of 1 gm., 1 received 1.5 gm., and 1 each received 4.0 and 4.5 gm. This amount is less than that used with the other deep mycoses.

Seven of the 8 patients exhibited some evidence of toxicity during the administration of amphotericin B. Azotemia is the most serious of the toxic effects encountered during the administration of this drug. Fever, nausea, emesis, and malaise, which accompany the administration of amphotericin B, can be reduced by the concomitant administration of judicious amounts of the corticosteroids. It also seems advisable to administer the drug every other day in order to lessen the untoward toxic effects (after authors' summary). —T. H. NOEHREN

Pneumoconiosis in Carbon Electrode Makers. A. J. WATSON*, J. BLACK, A. T. DOIG, and G. NAGELSCHMIDT. *Brit J Industr Med*, October, 1959, 16: 274–285.(*Western Infirmary, Glasgow, Scotland)

In the absence of adequate preventive measures, the manufacture of carbon electrodes is attended by a considerable dust hazard. The present paper is based on a study of the clinical, roentgenographic, and pathologic changes resulting from the inhalation of this dust, which is derived from crushed coke and anthracite. An account is given of the findings in a clinical survey of 15 men who had been employed for at least 10 years in manufacturing carbon electrodes. Four were suffering from complicated, and 5 from simple pneumoconiosis. In addition, the findings in 3 necropsied cases (2 complicated and 1 simple) are recorded in detail. Bacteriologic examination of the lungs and analysis of the lung dust was carried out in 2 cases of complicated pneumoconiosis.

It is shown that carbon-electrode makers may develop simple pneumoconiosis with focal emphysema, and that this may be complicated by massive fibrotic lesions. Both the simple and the complicated pneumoconiosis are indistinguishable from the corresponding conditions in other coal workers. Quartz was almost entirely absent from the lung dust of 2 necropsied cases with massive fibrosis and, in 1 of these cases, virulent tubercle bacilli were shown. The significance of these findings is discussed in relation to the etiology of progressive massive fibrosis. While it is evident that they are incompatible with the "silica" theory, they provide some limited support for the "tuberculosis" theory.—H. SIMON

Studies on the Nature and Etiology of Respiratory Disability in Witwatersrand Gold-Miners Free of Radiological Silicosis. M. R. BECKLAKE*, S. ZWI, and W. LUTZ. *Brit J Industr Med*, October, 1959, 16: 290–296. (*Council for Scientific and Industrial Research, Johannesburg, South Africa)

In an investigation into the cause of symptoms in gold miners who have no silicosis demonstrable on roentgenography, the relationship of dyspnea and various physiologic disturbances to dust exposure was examined. Forty-five subjects, aged 41 to 45 years, with normal chest films and long service underground were chosen for study from the population of past and present miners; only 34 men were tested, but the validity of the sample was checked by comparison with a similar group of miners reported previously. Each individual's dust exposure was measured in "particle-hours." A detailed history, including smoking habits, was followed by a clinical examination and a battery of lung function tests in each case.

No significant relationship was found between dyspnea and exposure or smoking. However, a negative correlation was observed between dust exposure and effort tests, implying that exercise capacity appeared best in those men whose dust exposure was greatest. Possible reasons for this finding are discussed. On the other hand, dyspnea did correlate with airway obstruction and hyperventilation on effort, indicating a physiologic

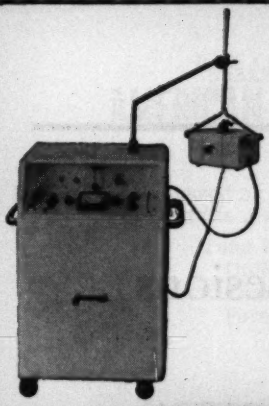
rather than a psychologic basis for the symptoms. Further, the higher incidence of cough, sputum, and rhonchi in the more disabled subjects suggested that chronic bronchitis might be the basis of their symptoms; but the cause of the bronchitis remains to be identified. It seems that dust exposure alone was not the cause, but the findings do not exclude the possibility of its being related to the occupation of mining with its stresses from inhalation of fumes and rapid changes in temperature, humidity, and altitude.—H. Simon

Population Studies of Chronic Respiratory Disease. A Comparison of Miners, Foundryworkers, and Others in Stavely, Derbyshire. I. T. T. Higgins*, A. L. Cochrane, J. C. Gilson, and C. H. Wood. *Brit J Industr Med*, October, 1959, 16: 255–268. (*Llandough Hosp., Nr. Penarth, Glamorgan, Wales)

Mortality and morbidity statistics suggest that miners and foundry workers are more prone to bronchitis than are other industrial workers, but it is not yet certain that this excess is due to occupational factors. The present investigation was designed to compare the prevalence of bronchitis and respiratory disability in a representative sample of miners, foundry workers, and other industrial groups living in Stavely, Derbyshire, a town of some 18,000 inhabitants, and to study some of the possible etiologic factors. A random sample of 776 men was used, stratified by age into 2 groups: 25 to 34, and 55 to 64 years; and by occupation into 4 groups: non-dusty, miners and ex-miners, foundry and ex-foundry workers, and other dusty jobs. Respiratory symptoms were recorded on a standard questionnaire (copy appended to publication) and the ventilatory capacity was assessed by means of the forced expiratory volume ($FEV_{0.75}$), and recorded as the indirect maximal breathing capacity (MBC).

Miners and ex-miners recorded a higher prevalence of respiratory symptoms and a lower mean MBC than did men who had worked in the dust-free operations. In the older age groups the differences were not large and were not statistically significant, but in the younger men the difference in the mean MBC was significant. Foundry and ex-foundry workers with a pure industrial history recorded a prevalence of symptoms similar to the men who had never worked in dusty occupations, and their mean MBC was only slightly and insignificantly lower. A higher prevalence of symptoms and a lower mean MBC was, however, recorded by the foundrymen who had also been exposed to other dusts or fumes, and the occupational histories suggested that such exposure was more likely than foundry work to account for the findings. The number of years spent on the coal-getting shift was used to assess the importance of exposure to coal dust. In the elderly miners without pneumoconiosis, there was a significant increase in the prevalence of breathlessness, accompanied by a reciprocal fall in the mean MBC with increasing years spent on the coal-getting shift; but in no other group was a consistent trend found.

In both age groups, the prevalence of respiratory symptoms was lower and the mean MBC higher in non-smokers than in smokers and ex-smokers. Heavy smokers (those smoking 15 gm. and more per day) recorded a higher prevalence of symptoms and a lower mean MBC than light smokers, and the values for ex-smokers approximated those of the non-smokers. The wives of the elderly men in the sample were studied to try to determine how far the apparently high rates of bronchitis shown by the national mortality statistics are attributable to social factors. The findings suggested that the wives of the men who worked in dusty jobs had a somewhat higher prevalence of cough and/or sputum and of chest illness during the past 3 years than did the wives of those who had worked only in dust-free occupations.—H. Simon



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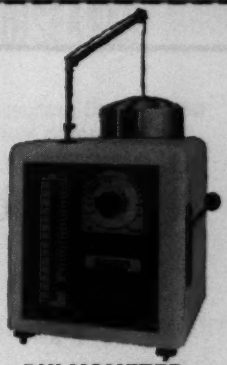

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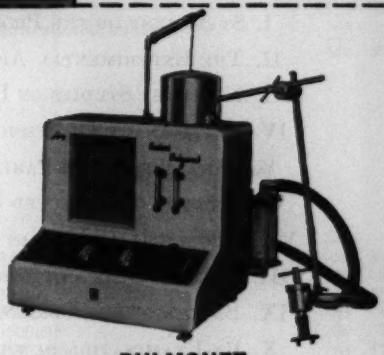

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No information	17	3.89
No age given	1	.22
30–34	10	2.29
35–39	55	12.61
40–44	58	13.30
45–49	50	11.46
50–54	51	11.69
55–59	37	8.48
60–64	35	8.02
65 and over	36	8.25
	350	80.21
Institutional subscribers	86	19.72
	436	99.93

POPULATION

	No.	%
Under 10,000	56	12.84
10–25,000	39	8.94
25–50,000	44	10.10
50–100,000	38	8.71
100–200,000	32	7.33
200–500,000	48	11.00
500–1,000,000	39	8.94
Over 1,000,000	54	12.38
	350	80.24
Institutional subscribers	86	19.72
	436	99.96

BREAKDOWN OF INSTITUTIONAL SUBSCRIPTIONS

Federal Hospital		General Hospital		Special Hospital		Laboratory		Medical Schools		College University		Society Library		Population
3		2		5		2		0		1		0		Under 10,000
3		2		1		0		0		1		0		10–25,000
0		2		4		0		3		2		1		25–50,000
1		1		1		0		1		2		2		50–100,000
2		0		1		1		0		0		2		100–200,000
3		6		0		0		0		1		4		200–500,000
4		3		2		0		0		1		0		500–1,000,000
2		4		1		1		2		0		6		Over 1,000,000
18	4.12%	20	4.58%	15	3.44%	4	.91%	6	1.37%	8	1.82%	15	3.44%	86 19.72%

Percentages figured only to second decimal point

See inside front cover for subscription rate

MANY CANCERS ARE CURABLE . . . NOW. These are words of hope for the thousands of cancer patients who see their physicians *in time.*

Tremendous gains can be made . . . *now* . . . in three of the most common cancer sites: breast, cervix, rectum. The annual health checkup can often detect early cancers in these sites at a time when *presently available* methods of treatment can effect many more cures than are being achieved today.

The American Cancer Society, therefore, in its broad public education program, emphasizes the importance of annual physical examinations for all adults.

Together an alerted public and the medical profession can win a major victory over cancer . . . *now.*

AMERICAN CANCER SOCIETY

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Diagnostic Reagents for the Determination of

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Bacto-Streptolysin O Reagent is a standardized reagent recommended as an aid in the diagnosis of Rheumatic Fever, Glomerulonephritis and other diseases associated with Group A streptococcal infections.

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Prothrombin determinations require accurately prepared interstandardized reagents: Bacto-Thromboplastin, Bacto-Prothrombin 2 Stage Reagent, Bacto-Prothrombin Free Beef Plasma, Bacto-Prothrombin Free Rabbit Plasma, Bacto-AC Globulin, Bacto-Sodium Chloride 0.85%, Bacto-Sodium Oxalate Solution 0.1 Molar, Bacto-Calcium Chloride Solution 0.02 Molar.

LIVER DISEASE

Differential diagnosis of hepatic disturbances is aided by standardized reagents: Bacto-Cephalin Cholesterol Antigen, Bacto-Thymol Turbidity Reagent, Bacto-Kingsbury Turbidity Standards, Bacto-Thromboplastin and other Prothrombin Reagents.

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NATIONAL TUBERCULOSIS ASSOCIATION 1904–1954

A Study of the Voluntary Health Movement in the United States

by Richard Harrison Shyrock, Ph.D.

W. H. Welch Professor of the History of Medicine, and Director of the Institute of the History of Medicine, The Johns Hopkins University.

342 pages, illustrated $3.50

This book would be a valuable addition to any medical library. It should be on the reference list of every medical school; and on the desk of all private physicians interested in the problems of tuberculosis.

Not only does it supply a historical background for the antituberculosis movement in the United States, but it affords enlightening orientation to the entire voluntary health movement with the National Tuberculosis Association as the principal illustration.

The three overlapping areas of the Association's activities—medical science and practice, public health, and social welfare—are presented here by an able medical historian who knows how to give facts in a clear and interesting way.

Among the chapters are: Medical Background; Social Background; Tuberculosis in the United States, 1904; Founding of the National Tuberculosis Association; Arousing the Public, 1904–1908; Growth of the Tuberculosis Movement, 1904–1920; Administrative Developments and Reorganization; Problems and Policies, 1908–1922; Widening Contracts and Activites, 1917–1922; Foreign Contacts, War, and the Emergence of a New Era; The Research Program, 1940–1954; Chemotherapy and the Climax of Fifty Years of Service.

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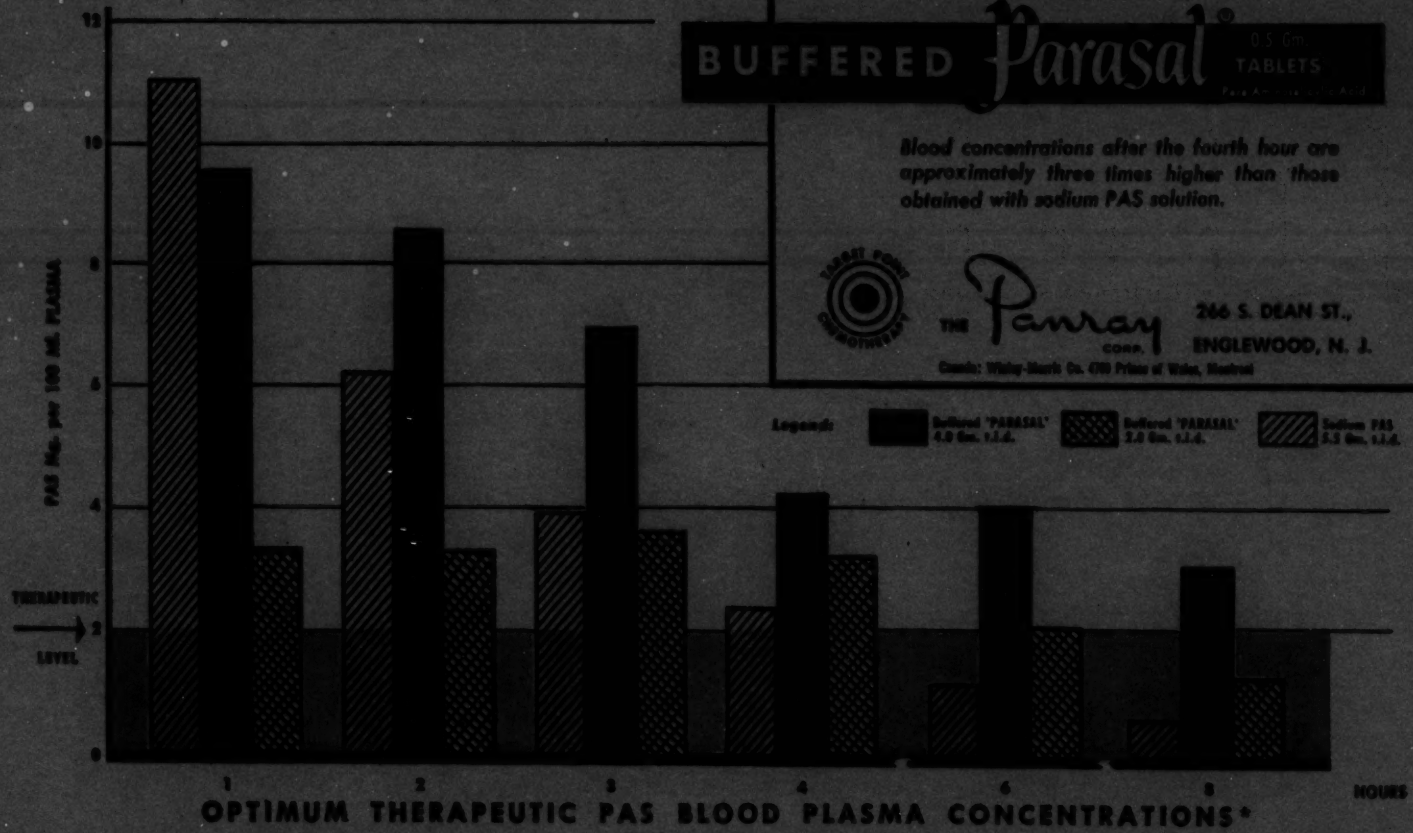

OPTIMUM THERAPEUTIC PAS BLOOD PLASMA CONCENTRATIONS*

Ref.: Deak, E. N. & Vitagliano, O.R.: A. Rev. Tuber. & Pul. Dis.: 72,540-7 (Oct. '55)

*based on multiple dosages, levels determined after 2nd dose of day.

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